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(71) Applicant(s)
QIAGEN Gaithersburg, Inc.

(72) Inventor(s)
Mallonee, Richard; Qiu, Peter

(74) Agent / Attorney
Davies Collison Cave, Level 14 255 Elizabeth Street, Sydney, NSW, 2000

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(71) Applicant (for all designated States except US): **QIAGEN GAITHERSBURG, INC.** [US/US]; 1201 Clopper Road, Gaithersburg, Maryland 20878 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MALLONEE, Richard** [US/US]; 7112 Wardman Road, Baltimore, Maryland 21212 (US). **QIU, Peter** [US/US]; 11407 Royal View Court, Gaithersburg, Maryland 20878 (US).

(74) Agent: **MCBEE, Susan**; 920 Massachusetts Ave., N.W., Suite 900, Washington, District of Columbia 20001 (US).

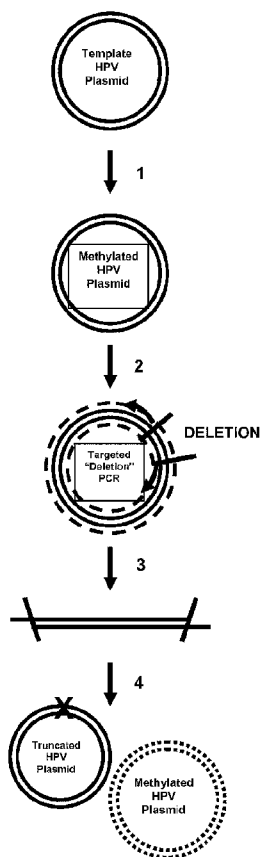
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(54) Title: FAST RESULTS HYBRID CAPTURE ASSAY AND ASSOCIATED STRATEGICALLY-TRUNCATED PROBES

FIG. 19

(57) Abstract: Strategically truncated probes specific for high-risk HPV nucleic acids, and methods for making and using the same, are disclosed herein. The disclosed probes, and methods of use thereof, permit fast and reliable detection of human papillomavirus in clinical samples without significant cross-reaction.





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FAST RESULTS HYBRID CAPTURE ASSAY AND ASSOCIATED STRATEGICALLY-TRUNCATED PROBES

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Patent Application
5 Number 61/347,941, filed on May 25, 2010, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

[0002] The present invention relates to methods, reagents, high throughput systems, and
10 kits for determining the presence of a nucleic acid in a sample.

2. BACKGROUND OF THE INVENTION

[0003] The detection and characterization of specific nucleic acid sequences and
sequence changes have been utilized to detect the presence of viral or bacterial nucleic acid
15 sequences indicative of an infection, the presence of variants or alleles of mammalian genes associated with disease and cancers, and the identification of the source of nucleic acids found in forensic samples, as well as in paternity determinations.

[0004] For example, the RNA or DNA for many microorganisms and viruses have been
isolated and sequenced. Nucleic acid probes have been examined for a large number of
20 infections. Detectable nucleic acid sequences that hybridize to complementary RNA or DNA sequences in a test sample have been previously utilized. Detection of the probe indicates the presence of a particular nucleic acid sequence in the test sample for which the probe is specific. In addition to aiding scientific research, DNA or RNA probes can be used to detect the presence of viruses and microorganisms such as bacteria, yeast and protozoa as well as genetic mutations
25 linked to specific disorders in patient samples.

[0005] Nucleic acid hybridization probes have the advantages of high sensitivity and
specificity over other detection methods and do not require a viable organism. Hybridization
probes can be labeled, for example with a radioactive substance that can be easily detected, or
with biochemical markers such as, for example, biotin, that allows for their capture and
30 detection. Highly sensitive strategically-truncated probes may also be constructed by eliminating

sequence regions exhibiting cross-reactive with undesirable or unwanted regions. Nucleic acid molecules may also be captured by a first antibody that is specific to DNA hybrids, wherein the hybrids may comprise DNA-RNA hybrids, DNA-DNA hybrids or RNA-RNA hybrids. The hybrids may subsequently be detected by a second, labeled, antibody that may be, for example, labeled with a biochemical marker such as alkaline phosphatase or any other marker capable of detection.

[0006] As nucleic acid sequence data for genes from humans and pathogenic organisms accumulates, the demand for fast, cost-effective, and easy-to-use tests increases. There is a need to provide novel and effective methods, compositions, and kits for determining a target nucleic acid in a sample faster and more. The methods and assays of the present invention meet these needs and may be used in high throughput automated systems. In another aspect, the methods and assays may be implemented in partially automated systems.

BRIEF SUMMARY OF THE INVENTION

[0006a] According to a first aspect of the present invention there is provided a polynucleotide probe at least 1 kilobase in length, comprising at least one sequence having from 70% to 100% complementarity to at least 100 contiguous bases of each of L1, L2, E1, E2, E4, E6, and E7 of a high risk HPV nucleic acid selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 17, SEQ ID NO: 104 to SEQ ID NO: 110, SEQ ID NO: 116 and complements thereof, wherein said polynucleotide probe does not contain any sequences of at least 100 contiguous bases having 70% to 100% complementarity to at least 100 contiguous bases of a low risk HPV nucleic acid of the group consisting of SEQ ID NO: 18 to SEQ ID NO: 43 and complements thereof.

[0006b] According to a second aspect of the present invention there is provided a kit comprising at least one polynucleotide probe of the first aspect.

[0006c] According to a third aspect of the present invention there is provided a method for generating a polynucleotide probe specific for a high risk HPV nucleic acid, said method comprising:

- a) providing a template nucleic acid comprising a sequence at least 1 kilobase in length sharing from about 70% to 100% identity with at least a portion of the high risk HPV nucleic acid;
- b) comparing the sequence of the template nucleic acid with at least one low risk nucleic acid; and

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- c) truncating the template nucleic acid to remove any regions of the template nucleic acid that share from about 70% to 100% identity over 100 contiguous base pairs with a region of the low risk HPV nucleic acid.

[0006d] According to a fourth aspect of the present invention there is provided a polynucleotide probe generated according to the method according to the third aspect.

[0006e] According to a fifth aspect of the present invention there is provided a nucleic acid probe set generated by fragmenting a polynucleotide probe of the first aspect.

[0006f] According to a sixth aspect of the present invention there is provided a kit comprising a nucleic probe set of the fifth aspect.

[0006g] According to a seventh aspect of the present invention there is provided a method for determining the presence of a target nucleic acid in a sample, the method comprising:

- a) hybridizing at least one polynucleotide probe according to the first aspect and the fourth aspect to the target nucleic acid molecule to form a double-stranded nucleic acid hybrid;
- b) capturing the double-stranded nucleic acid hybrid on a support, wherein the support comprises a first antibody specific for a double-stranded nucleic acid hybrid; and
- c) detecting the target nucleic acid molecule.

[0007] In one aspect, the present disclosure relates to polynucleotide probes at least 1 kilobase in length wherein the polynucleotide probe is capable of hybridizing to a high risk HPV nucleic acid, but does not cross-react with a low risk HPV nucleic acid. In one aspect, the polynucleotide probe comprises at least one sequence having from 70% to 100% complementarity to at least 100 contiguous bases of each of L1, L2, E1, E2, E4, E6, and E7 of a high risk HPV nucleic acid, wherein said polynucleotide probe. In a further aspect, the polynucleotide probe does not contain any sequences of at least 100 contiguous bases which have about 70% to 100% complementarity to at least 100 contiguous bases of a low risk HPV nucleic acid. In another aspect, the polynucleotide probe does not comprise any sequences having about 70% to 100% identity to SEQ ID NO: 44 to SEQ ID NO: 57, SEQ ID NO: 111 to SEQ ID NO: 115, or a complement thereof. In yet a further aspect, the polynucleotide probe is specific for a high risk HPV nucleic acid selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 17 and SEQ ID NO: 104 to SEQ ID NO: 110, SEQ ID NO: 116, or a complement thereof. In another aspect, the polynucleotide probe comprises a sequence having about 70% to 100% identity to a sequence selected from the group consisting of SEQ ID NO: 97 to SEQ ID NO: 103 or a complement thereof. In yet another aspect, the polynucleotide probe consists

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essentially of or consists of a sequence selected from the group consisting of SEQ ID NO: 97 to SEQ ID NO: 103 or a complement thereof.

[0008] In an aspect, the present disclosure provides for a nucleic acid comprising a sequence at least 1 kilobase in length sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 116, SEQ ID NO: 117 or a complement thereof, with the proviso that said nucleic acid does not comprise any sequences sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity with SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, or a complement thereof.

[0009] The present disclosure also provides for a probe set of one or more probes comprising, consisting essentially of, or consisting of a nucleic acid sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 104, SEQ ID

NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 116; SEQ ID NO: 117, or a complement thereof, with the proviso that said nucleic acid does not comprise any sequences sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity with SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, or a complement thereof.

[0010] In an aspect, the disclosure provides for a method for determining the presence of a target nucleic acid molecule in a sample including:

- a) suspending the sample in a collection medium;
- b) releasing target nucleic acid molecules from the sample into the collection medium;
- c) converting double-stranded target nucleic acid molecules to single-stranded target nucleic acid molecules;
- d) contacting one or more polynucleotide probes with the single-stranded target nucleic acid molecules under conditions that allow the polynucleotide probes and target single-stranded target nucleic acid molecules to hybridize forming double-stranded nucleic acid hybrids;
- e) capturing the double-stranded nucleic acid hybrids;
- f) separating the double-stranded nucleic acid hybrids from un-bound single-stranded target nucleic acid molecules; and
- g) detecting the double-stranded nucleic acid hybrids, thereby indicating the presence of the target nucleic acid.

[0011] In an aspect, the polynucleotide probe is a strategically-truncated probe specific for a high risk HPV nucleic acid, wherein the deleted portion shares high sequence identity or cross reactivity to a HPV low risk type. In an aspect, the HPV high risk strategically-truncated probe is specific for or capable of hybridizing to one or more HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 and the deleted portion exhibits cross reactivity or specificity with one or more of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the deleted portion of the probe comprises a sequence sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ

ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57. In another aspect, the polynucleotide probes are nucleic acid sequences sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, or a complement thereof.

[0012] Another aspect relates to the rapid detection of target nucleic acid molecules in a sample using the probes disclosed herein. The detection method may be automated, either fully automated, or partially automated – in other words requiring some human input.

[0013] Another aspect relates to the detection of target nucleic acid molecules in multiple samples at the same time or within a very short period of time, for example in a machine or a series of machines, using the probes disclosed herein.

[0014] Another aspect relates to a kit for the detection of a target nucleic acid molecule in a sample comprising the probes disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **Fig. 1** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV33. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 5 (including vector sequences) and SEQ ID NO: 104 (HPV sequences only).

[0016] **Fig. 2** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV39. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 7 (including vector sequences) and SEQ ID NO: 105 (HPV sequences only).

[0017] **Fig. 3** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV52. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 10 (including vector sequences) and SEQ ID NO: 106 (HPV sequences only).

[0018] **Fig. 4** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV56. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 11 (including vector sequences) and SEQ ID NO: 107 (HPV sequences only).

[0019] **Fig. 5** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV58. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 12 (including vector sequences) and SEQ ID NO: 108 (HPV sequences only).

[0020] **Fig. 6** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV66. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 14 (including vector sequences) and SEQ ID NO: 109 (HPV sequences only).

[0021] **Fig. 7** exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV68. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation. Template sequences can be found at SEQ ID NO: 15 (including vector sequences) and SEQ ID NO: 110 (HPV sequences only).

[0022] Fig. 8 exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV26 (SEQ ID NO: 116). Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation.

5 [0023] Fig. 9 exhibits the sequence for an exemplary template which can be used to generate strategically-truncated probes specific for HPV73. Underlined text indicates binding sites for the deletion primers. Hatched underlining indicates overlapping sites for the deletion primers. Lower-case text indicates sequences selected for truncation.

10 [0024] Fig. 10 exhibits a restriction map of the HPV26XX strategically-truncated probe and its sequence (SEQ ID NO: 117). Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

15 [0025] Fig. 11 exhibits a restriction map of the HPV33X strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

20 [0026] Fig. 12 exhibits a restriction map of the HPV39XX strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

25 [0027] Fig. 13 exhibits a restriction map of the HPV52X strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

[0028] Fig. 14 exhibits a restriction map of the HPV56XX strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

30 [0029] Fig. 15 exhibits a restriction map of the HPV58X strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer

overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

[0030] **Fig. 16** exhibits a restriction map of the HPV66XX strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. Note that “****” indicates the site at which the primers pHPV66XF1 (SEQ ID NO: 76) and pHPV66XR1 (SEQ ID NO: 77) would bind in the template. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

[0031] **Fig. 17** exhibits a restriction map of the HPV68XX strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

[0032] **Fig. 18** exhibits a restriction map of the HPV73XX strategically-truncated probe and its sequence. Deletion primer binding sites are indicated by single underlining, with primer overlap indicated by hatched underlining. The promoter site is indicated by bolded, italicized, and double underlined text. Shaded text indicates probe sequences.

[0033] **Fig. 19** exhibits a sample schematic for generating truncated HR-HPV probes. As show in Fig. 19, a plasmid comprising the deletion probe is generated according to the following steps: 1. The target HPV template plasmid is methylated. 2. Plasmids are amplified, minus the targeted deletion, using two primers with overlapping tails (deletion primers). 3. The resulting PCR products are linear double stranded DNA minus the segment targeted for deletion. 4. The PCR plasmid mixture is transformed into wild-type *E.coli*. The host cell circularizes the deleted plasmid and expressed *McrBC* nuclease digests the original methylated target plasmid. This leaves only the unmethylated truncated plasmid which is replicated. Linear probes may then be generated from the truncated plasmid, such as by the exemplary method at Fig. 31.

[0034] **Fig. 20** demonstrates a sample schematic for generating the HPV 26XX strategically-truncated probe. 1. A methylated plasmid bearing the HPV26 sequence according to GenBank accession number X74472 was amplified with the primer pair p26F and p26R1 set forth at A to generate single strategically-truncated plasmid HPV26X, which is then cloned. The binding site for each primer is indicated on plasmid HPV 26 v2. 2. After cloning, HPV26X is

amplified using the primer pair p26XF3 and p26XR3 to generate HPV26XX. The binding site for each primer is indicated on plasmid HPV 26X.

[0035] **Fig. 21** exhibits a restriction map and a sample digestion of HPV26XX strategically-truncated plasmid.

5 [0036] **Fig. 22** demonstrates a sample schematic for generating the HPV 33X strategically-truncated plasmid.

[0037] **Fig. 23** demonstrates a sample schematic for generating the HPV 52X strategically-truncated plasmid.

[0038] **Fig. 24** demonstrates a sample schematic for generating the HPV 58X
10 strategically-truncated plasmid.

[0039] **Fig. 25** demonstrates a sample schematic for generating the HPV 39XX strategically-truncated plasmid.

[0040] **Fig. 26** demonstrates a sample schematic for generating the HPV 56XX strategically-truncated plasmid.

15 [0041] **Fig. 27** demonstrates a sample schematic for generating the HPV 66XX strategically-truncated plasmid.

[0042] **Fig. 28** demonstrates a sample schematic for generating the HPV 68XX strategically-truncated plasmid.

[0043] **Fig. 29** demonstrates a sample schematic for generating the HPV 73XX
20 strategically-truncated plasmid.

[0044] **Fig. 30** demonstrates the results of a sequence alignment between high risk and low risk HPV-types using Vector NTI 10.3.0 software (Invitrogen Corp., Carlsbad, CA). Shaded cells indicate sequences having a high risk of cross-reactivity. Sequences having from about 70% to 100% sequence identity are deemed to have a high risk of cross-reactivity. Sequences
25 having from about 75% to 100% sequence identity are deemed to have a moderately high risk of cross-reactivity. Sequences having from about 80% to 100% sequence identity are deemed to have very high risk of cross-reactivity.

[0045] **Fig. 31** demonstrates an exemplary scheme for generating linearized probes from truncated plasmids. To introduce uniformity, each template is cloned from L2 to L1 as XhoI/Not
30 I fragments with a promoter sequence (ctcactataggcggaattgg) (SEQ ID NO: 96). Cloned in this manner, all of the HPV nucleic acids are in the same orientation and can be linearized with one

enzyme, *XhoI*, prior to RNA transcription. The T7 polymerase initiates RNA transcription at the promoter sequence, which terminates at the end of L1 to produce the full length probe without including any of the vector sequence. The sequence of each linearized RNA probe generated in this manner is set forth at SEQ ID NO: 97–105.

5 DETAILED DESCRIPTION OF THE INVENTION

[0046] The present disclosure includes methods, compositions, reagents, systems, and kits for rapidly determining the presence of a nucleic acid molecule in a sample. The methods, compositions, reagents, systems, and kits may be used for clinical diagnostic purposes, including but not limited to the detection and identification of pathogenic organisms and the detection of a genetic predisposition to a particular disease.

10 [0047] In an aspect, nucleic acid probes are disclosed, said probes having the ability to detect a first HPV nucleic acid without substantially cross-reacting with a second HPV nucleic acid.

[0048] In one aspect, said nucleic acid probes are generated by a method comprising: a) providing a template nucleic acid comprising a sequence at least 1 kilobase in length sharing from about 70% to 100% identity with the first nucleic acid; b) comparing the sequence of the template nucleic acid with the sequence of the second nucleic acid; and c) truncating any regions of the template nucleic acid that share from about 70% to 100% identity with a region of the second nucleic acid.

20 [0049] In an aspect, the present disclosure provides for a nucleic acid comprising a sequence at least 1 kilobase in length sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106,

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SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 116, or SEQ ID NO: 117, or a complement thereof, with the proviso that said nucleic acid does not comprise any sequences sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity with SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, or a complement thereof.

[0050] The present disclosure also provides for a probe set of one or more probes comprising, consisting essentially of, or consisting of a nucleic acid sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 116, or SEQ ID NO: 117, or a complement thereof, with the proviso that said nucleic acid does not comprise any sequences sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity with SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, or a complement thereof.

[0051] In a further aspect, the probe set comprises at least one nucleic acid probe comprising, consisting essentially of, or consisting of a sequence sharing 70% or more; 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity with a sequence selected from the group consisting of SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID

NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 117, or a complement thereof.

[0052] In an aspect, the present disclosure provides for a nucleic acid comprising, consisting essentially of, or consisting of a sequence sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 117, or fragments or complements thereof.

[0053] In one aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample. The method includes:

- a) suspending the sample in a collection medium comprising a detergent;
- b) denaturing the target nucleic acid molecule;
- c) contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize, thereby forming a double-stranded nucleic acid hybrid;
- d) capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a double-stranded nucleic acid hybrid/solid support complex;
- e) separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid;
- f) conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex; wherein the second antibody is labeled with a detectable marker;
- g) washing the double-stranded nucleic acid hybrid/solid support antibody complex with a wash buffer comprising a detergent; and
- h) detecting the label on the second antibody wherein the detecting indicates the presence of the target nucleic acid molecule.

[0054] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a

collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, and capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid.

[0055] In an aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid and separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid.

[0056] In an aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a double-stranded nucleic acid hybrid/solid support complex; and separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid; conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex.

[0057] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a double-stranded nucleic acid

hybrid/solid support complex; and separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid; conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex; wherein the second antibody is labeled with a detectable marker; and washing the double-stranded nucleic acid hybrid/solid support antibody complex with a wash buffer comprising a detergent.

[0058] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample, the method comprising:

- a) suspending the sample in a collection medium comprising a detergent;
- b) denaturing the target nucleic acid molecule in the sample;
- c) forming a double-stranded nucleic acid hybrid by contacting at least one polynucleotide probe with the target nucleic acid molecule;
- d) forming a double-stranded nucleic acid hybrid-support complex by capturing the double-stranded nucleic acid hybrid on a support, wherein the support comprises a first antibody;
- e) forming a double-stranded nucleic acid hybrid-support-second antibody complex by contacting the double-stranded nucleic acid hybrid-support complex with a second antibody, wherein the second antibody is labeled with a detectable marker;
- f) washing the double-stranded nucleic acid hybrid-support-second antibody complex with a wash buffer; and
- g) detecting the marker on the second antibody wherein the detecting indicates the presence of the target nucleic acid molecule.

[0059] In an aspect, the polynucleotide probes used in the methods described herein are HPV high-risk strategically-truncated probes. In another aspect, the polynucleotide probes are HPV high risk probes and the deleted portion exhibits high sequence identity or cross reactivity to a HPV low risk type. In an aspect, the HPV high risk probe is specific for or capable of hybridizing to one or more of HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 and the deleted portion shares cross reactivity or specificity with one or more of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the polynucleotide probes comprise, consist, or consist essentially of a sequence sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89,

SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, or SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 117, or fragments or complements thereof.

5 **[0060]** In one aspect, the solid support includes a modified paramagnetic bead that is coated or has attached thereto a first antibody immunospecific for double-stranded hybrid nucleic acids. A magnetic field can be used to separate the double-stranded nucleic acid-magnetic bead-antibody complex from non-bound nucleic acid.

10 **[0061]** In an aspect, the method does not include a sample pre-treatment step. For example, the detergent-based collection medium allows for reduced sample preparation time which, in turn, can lead to accelerated detection of target nucleic acid molecules. The sample can be analyzed by methods, assays, or the apparatus of the disclosure in a direct-to-assay manner. In an example, purification steps are not performed on the sample prior to evaluation using assays of the disclosure. In an aspect, crude lysate is directly analyzed by the methods,
15 assays, or the apparatus of the disclosure. In another aspect, the sample does not undergo a target amplification step.

20 **[0062]** One aspect relates to a method of diagnosing cancer by utilizing methods, kits, assays, and the apparatus provided herein. In one aspect, cervical cancer is detected by identifying nucleic acid molecules associated with HPV and HPV variants. In another aspect, cervical intraepithelial neoplasia (CIN) can be screened for using methods, kits, assays, and the apparatus provided herein. The detected cancer can be subsequently treated after being diagnosis by the methods, kits, assays, and the apparatus provided herein. In an aspect, the diagnosed cancer is cervical cancer and variants thereof.

25 **[0063]** In an aspect, the disclosure provides for a composition comprising
(a) a biological sample suspended in about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide; and

(b) one or more polynucleotide probes.

30 **[0064]** In an aspect, the disclosure provides for a composition comprising

(a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide;

- 5 (b) one or more polynucleotide probes; and
 (c) a first antibody.

[0065] In an aspect, the disclosure provides for a composition comprising

 (a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75
10 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide;

- (b) one or more polynucleotide probes;
 (c) a first antibody; and
 (d) a second antibody.

15 **[0066]** In an aspect, the disclosure provides for a composition comprising

 (a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide;

- 20 (b) one or more polynucleotide probes;
 (c) a support coated with a first antibody; and
 (d) a second antibody.

[0067] In an aspect, the disclosure provides for a composition comprising

 (a) a biological sample suspended in a collection medium, wherein the collection
25 medium comprises at least one detergent;

- (b) a denaturation reagent;
 (c) one or more polynucleotide probes;
 (d) a support coated with a first antibody; and
 (e) a second antibody labeled with a detectable marker.

30 **[0068]** In an aspect, the polynucleotide probes used in the compositions described herein are HPV high-risk strategically-truncated probes. In another aspect, the polynucleotide probes

are HPV high risk probes and the deleted portion exhibits high sequence identity or cross reactivity to a HPV low risk type. In an aspect, the HPV high risk probe is specific for or capable of hybridizing to one or more of HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 and the deleted portion shares cross reactivity or specificity with one or more of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the polynucleotide probes are nucleic acid sequences sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, or SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 117, or fragments or complements thereof.

[0069] As used herein, the phrase “high risk HPV” refers to HPV types associated with an elevated risk of developing cancer, which are well known to a person having ordinary skill in the art.

[0070] As used herein, the phrase “low risk HPV” refers to HPV types that are not associated with an elevated risk of developing cancer, which are well known to a person having ordinary skill in the art.

[0071] In an aspect, any of the above compositions may be used may be used with any of the collection mediums described herein. In an aspect, the biological sample in the above compositions is a cervical cell sample or a human cervical cell sample. In another aspect, the nucleic acid molecules in the biological sample are denatured. The biological sample in the above compositions can exhibit stability when stored in the collection medium for at least 21 days at 33°C. In an aspect, the second antibody is labeled with a detectable marker.

Biological Sample

[0072] Methods of the present disclosure may be used to detect the presence of a target nucleic acid molecule from samples, including, without limitation, a specimen or culture (*e.g.*, cellular, microbiological and viral cultures) including biological and environmental samples. Biological samples may be from an animal, including a human, fluid, solid (*e.g.*, stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Environmental samples include

environmental material such as surface matter, soil, water and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items.

[0073] The biological samples may include, but are not limited to, cervical epithelial cells (*e.g.*, a sample obtained from a cervical swab), adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen. The sample may comprise a double-stranded nucleic acid molecule or may comprise a single-stranded nucleic acid molecule. If a double-stranded nucleic acid molecule is present, it may be prepared for hybridization analysis by a variety of methods known in the art, *e.g.*, using alkali, using proteinase K/SDS, chaotropic salts. The process of preparing a double-stranded nucleic acid molecule for hybridization analysis generally involves converting it into a single-stranded nucleic acid molecule. This process is generally known as denaturation. However, it is also contemplated that a double-stranded nucleic acid molecule may be detected without denaturation, *e.g.*, through a triple-stranded construct.

[0074] The target nucleic acid molecule in a sample can be DNA or RNA or both DNA and RNA. The target nucleic acid molecule can be contained within a larger nucleic acid molecule. Detection of either the target nucleic acid molecule or the larger nucleic acid molecule containing the target nucleic acid molecule is contemplated by this disclosure.

[0075] The biological sample may comprise cervical cells, especially human cervical cells. The sample can be collected with any method or device known in the art, including a chemically inert collection device such as a DACRON tipped swab. Other acceptable collection devices may be used including, but not limited to, cotton swab, cervical brush, flocked swab (a swab shaped like a DACRON swab but made with nylon fibers enabling collection of more cells and easier release of cells), cervical broom, mini broom, lavage, or any collection device often used in Pap smear testing.

[0076] In an aspect, the methods include collecting a sample from a woman at least 30 years of age. The method can also include collecting a sample from a woman at least 30 years via a Pap smear or comparable test. The sample collected by the Pap smear or comparable test can be a cervical cell sample.

[0077] Once the sample is collected, it may be placed in a sample tube. The tube can be sealed to prevent contamination. The collection device (swab, brush, etc.) may further contain a

mechanism by which it can be moved once it is inside the sample tube. In one aspect, the collection device contains an insert that can be moved using a magnet. In one aspect, this insert comprises a metal. In another aspect, this insert comprises a magnetic material. Magnetic material includes paramagnetic, ferromagnetic, and diamagnetic materials. One advantage of moving the collection device once it is inside the sample tube is to avoid the collection device from making contact with any sample extraction or sample detection devices. Examples of a sample extraction device include pipettes, automated pipettor, and pipette tips. Examples of sample detection devices include probes and probe tips.

Sample Tube

[0078] Any type of sample tube may be used. The sample tube may be closed or sealed to minimize contamination. The closure may be permanent or removable. Examples of removable closures include snap caps, screw caps, rubber septa, foil, and film. The closure may contain one or more openings or perforations, which when pierced may be re-sealable. One advantage of a closure that contains such openings or perforations is that the closure is not rendered ineffective when pierced by, for example, a sample extraction device or sample detection device. Once the sample extraction device or sample detection device is removed, the closure re-seals, thereby minimizing contamination.

Storage of the Biological Sample

[0079] Once the sample is in the sample tube, the sample may be stored by drying it with a substrate, or in a preservative medium, or both. Desiccation is accomplished by pressure drying or drying with chemicals. This removes most of the water and is suitable for long-term stability. Alternatively, the sample may be lyophilized (freeze-dried) with a substrate like trehalose to ensure stability of the sample.

[0080] Another possibility is that the sample may be stored by suspending in a preservative medium, known and apparent to one of skill in the art. The purpose of the preservative medium is to protect biological components that can degrade. For instance, the sample cells, the probe mixture, the antibody: bead complex used in the capture step, and the secondary antibody used in the detection step are all susceptible to degradation. A preservative medium at the initial step of collection ideally provides sample stability and integrity and can affect downstream steps in the process of nucleic acid capture and detection.

Collection Medium

[0081] In an aspect, the sample may be collected and stored in a collection medium. The collection medium has several functions including as a preservative medium to preserve nucleic acids and inhibit nucleases to prevent degradation of nucleic acids prior to analysis. In one aspect, the collection medium contains at least one detergent. In another aspect, the collection medium contains at least two detergents, at least three detergents, or at least four detergents. In an aspect, each of the detergents is different. In another aspect, the detergent-based collection medium comprises two different detergents, one which is able to control background signal and another detergent that improves magnetic bead behavior, for example, migration through a viscous sample, collection, *i.e.*, how well the magnetic beads gather together at the bottom of the sample well, and retention, *i.e.*, how well the magnetic beads stay in place when a supernatant is either removed from a container containing the sample.

[0082] In an aspect, heat is employed during the hybridization, capture, and detection steps of the assay. Even with detergent and the application of heat, antibodies used in the assay remain functional.

[0083] The detergent-based collection medium may comprise, consist essentially of, or consist of one, two, three, or four or more detergents. Detergents are known in the art and may include, but are not limited to, cationic detergents such as but not limited to cetyl pyridinium bromide, cetyltrimethylammonium bromide (collectively known as cetrimonium compounds) and alkylbenzyltrimethylammonium chlorides (collectively known as benzalkonium compounds), and alkyl-trimethyl-ammonium salts; anionic detergents such as, but not limited to, sodium dodecyl sulfate (SDS), and Sarkosyl; and non-denaturing detergents such as NP-40; and other detergents. NP-40 is also known as Tergitol-type NP-40, which is nonyl phenoxy polyethoxy ethanol. NP-40 is not powerful enough to break the nuclear membrane, but can break the plasma membrane. As such, it can be used to obtain the cytoplasmic contents of a cellular culture.

[0084] Other detergents and combination of detergents may be used, and their combination provides the ability to control background noise and improve magnetic bead behavior (when the solid support employed comprises magnetic beads). In certain aspects, one detergent is an anionic detergent and the second detergent is a nonanionic detergent. For example, in one aspect, the combination of non-ionic and anionic detergents helps to maintain

low-background noise. In an aspect, a detergent-based collection medium comprises an anionic detergent such as sodium deoxycholate, which controls background noise and NP-40, which may improve magnetic bead behavior.

[0085] The combination of these two types of detergents provides synergistic benefits beyond a simple combination of adding two detergents together: control of background noise, better bead behavior, and increased assay speed. The presence of these detergents (in the detergent-based collection medium) provides the ability to achieve faster assay results, but does not negatively impact the nucleic acid or capture antibody during downstream analytical steps.

[0086] In addition, the detergent-based collection medium improves removal of the specimen from the collection device as the sample is dissolved more easily. In addition, the detergent-based collection medium improves the homogeneity of the sample compared with other collection media such as but not limited to PRESERVACYT (uses a 40% methanol solution), STM (uses a chaotropic agent), and alcohol. The detergent-based collection medium also reduced sample viscosity after mixing (either manual or automated).

[0087] The concentration of NP-40 in the collection medium can range from about 0.5% to about 2.0%, from about 0.1% to about 1.0%, as well as any number within the recited ranges. In certain aspects, the NP-40 is present at a concentration from about 0.8% to about 1.5 %; from about 0.9% to about 1.2% and in certain aspects is about 1.0%. In another aspect, the NP-40 is present at a concentration from about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0%. The concentration of sodium deoxycholate in the collection medium can range from about 0.10% to about 0.40%, from about 0.20% to about 0.30%, as well as any number within the recited ranges. In one aspect, the concentration of sodium deoxycholate is about 0.10%, about 0.15%, about 0.20%, about 0.25%, about 0.30%, about 0.35%, or about 0.40%.

[0088] The detergent-based collection medium may comprise, consist essentially of, or consist of a buffer, two detergents, a chelator and a preservative. The buffer may be Tris-HCl in a concentration of from about 25 mM to about 75mM; from about 30 mM to about 60 mM; from about 40 mM to about 50 mM, and from about 45 mM to about 55 mM as well as any number within the recited ranges. The buffer may also be Tris-HCl in a concentration of about 25 mM,

about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, or about 75 mM.

[0089] Any preservative can be used and the choice can depend on factors such as desired functionality, minimization side-effects, cost, etc. Suitable preservatives include gentomycin, ProClin, dimersol, and sodium azide. The concentration of the preservative in the collection medium depends on factors such as the type of preservative, its efficacy, its side-effects, etc. For example, for sodium azide, the concentration of sodium azide can range from about 0.01% to about 0.1%, from about 0.025% to about 0.075%, and from about 0.04% to about 0.06%, as well as any number within the recited ranges. The preservative, for example, sodium azide, can also be present at about 0.01%, about 0.02%, about 0.03%, about 0.04%, 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.10%.

[0090] In one aspect the detergent-based collection medium comprises, consists essentially of, or consists of 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25 mM EDTA, 150 mM NaCl and 0.09% sodium azide. In another aspect the detergent-based collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide. In other aspects the detergent-based collection medium comprises, consists essentially of, or consists of about 0.8% to about 1.5% NP-40, about 0.20% to about 0.40% sodium deoxycholate, about 30 mM to about 60 mM Tris-HCl, about 20 mM to about 40 mM EDTA, about 100 mM to about 200 mM NaCl, and about 0.025% to about 0.075% sodium azide. In yet another aspect the detergent-based collection medium comprises, consists essentially of, or consists of about 0.9% to about 1.2% NP-40, about 0.20% to about 0.30% sodium deoxycholate, about 30 mM to about 60 mM Tris-HCl, about 20 mM to about 30 mM EDTA, about 100 mM to about 150 mM NaCl, and about 0.04% to about 0.06% sodium azide.

[0091] In an aspect, the collection medium comprises, consists essentially of, or consists of NP-40 and EDTA. In another aspect, the collection medium comprises, consists essentially of, or consists of NP-40, EDTA, and sodium azide. In one aspect, the collection medium comprises, consists essentially of, or consists of sodium deoxycholate, EDTA, and sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about NP-40, sodium deoxycholate, EDTA, and sodium azide. In an aspect, the collection medium

comprises, consists essentially of, or consists of NP-40, sodium deoxycholate, Tris-HCl, EDTA, and sodium azide.

[0092] In another aspect, the collection medium comprises, consists essentially of, or consists of 0.5% to about 2.0% NP-40 and 10 mM to about 50 mM EDTA. In another aspect, the collection medium comprises, consists essentially of, or consists of 0.5% to about 2.0% NP-40, 10 mM to about 50 mM EDTA, and about 0.01 % to about 0.10 % sodium azide. In one aspect, the collection medium comprises, consists essentially of, or consists of about 0.10% to about 0.40% sodium deoxycholate, 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide.

[0093] In an aspect, the collection medium is a non-chaotropic medium. That is, for example, the collection medium does not include a chaotropic medium or chaotropic salts. Without being limited, in an aspect, the collection medium does not include guanidine hydrochloride or urea. A potential advantage of using a non-chaotropic collection medium is better resuspension of a sample, more reproducible testing, and more uniform testing aliquots relative to a medium which includes a chaotropic medium or chaotropic salts.

[0094] An advantage of using a detergent-based collection medium is that it preserves the stability of the sample. A sample stored in a detergent-based collection medium as disclosed is stable for at least 31 days, and, when held at temperatures from 15°C to 33°C is stable for at least 21 days. In an aspect, a sample is stable when frozen in a detergent-based collection medium at -20°C for at least six months. In another aspect, a cervical cell sample is stable for at least 31 days, for at least 21 days when held at temperatures from 15°C to 33°C, and for at least 6 months in a detergent-based collection medium at -20°C.

[0095] A detergent-based collection medium also leads to improved assay performance under rigorous hybridization and capture conditions (for example, at temperatures between 65° - 75°) relative to collection medium containing a denaturant.

[0096] The presence of one, two, three, four or more detergents can reduce sample viscosity, which aids in the removal of the liquid phase from the magnetic beads, as well as aids in the mixing of samples.

[0097] In one aspect, a sample such as blood or an exfoliated cervical cell specimen can be collected and suspended in a detergent-based collection medium. The sample can be is collected with a chemically inert collection device such as a DACRON tipped swab. Any other suitable swab may be used such as nylon fiber swabs. The sample may be stored in a detergent-based collection medium, to prevent degradation of nucleic acids prior to analysis and to maintain stability of the sample.

[0098] Samples may be collected in other known collection mediums and then can be used in the methods described herein. Examples of other collection media include PRESERVCYT, SUREPATH, DIGENE Collection Medium ("DCM"), and STM (Sample/Specimen Transport Medium). Certain collection media are nucleic acid specific. For example DCM is not used when the target nucleic acid is RNA. Samples collected in some of these media may require processing before the nucleic acids in the samples can be detected and analyzed. Various methods of processing samples (also known as preparing the samples) are known in the art. For example, cervical cell samples collected for cytological analysis in medium such as PRESERVCYT may be combined with a detergent-based lysis buffer followed by the addition of magnetic beads comprising nucleic acid binding surfaces. In addition, other cell samples collected in other known commonly available collection mediums may be combined with a detergent-based lysis buffer followed by the addition of magnetic beads comprising nucleic acid binding surfaces.

[0099] In another aspect, the lysis buffer includes 150mM Tris-HCl (pH 8.0), 0.09% (w/v) Sodium Azide, 6% (v/v) Triton x-100, 300mM Diethanolamine (w/v), with a final pH of between 9.3 and about 9.5. In another yet another aspect, the lysis buffer includes between about 100 mM to about 200 mM Tris-HCl at between about pH 7.5 and about 8.5, between about 0.05% and about 0.10% (w/v) sodium azide, between about 2.5% to about 7.5% (v/v) Triton x-100, and between about 200mM to about 400mM Diethanolamine (w/v), with a final pH of between 9.0 and about 10.0.

Pre-treatment

[00100] In an aspect, the assay does not include a sample pre-treatment preparation step. In another aspect, the assay does not include sample pre-treatment preparation when a detergent-based collection medium is used. For example, a sample pre-treatment preparation is not
5 required when the detergent-based collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide. Any combination of the components is also contemplated.

10 [00101] In another aspect, the assay can include a pre-sample treatment preparation step when either PRESERVCYT or SUREPATH are used as the collection medium. The pre-treating may be done manually or may be automated.

[00102] One example of an automated pre-treatment machine is a Pre-Analytic System (PAS) instrument that is adapted to process biological samples, including liquid based cytology
15 (LBC) samples into standard 96-well plates containing the extracted sample nucleic acid. In the PAS, samples are processed in a strip of eight test tubes, called the extraction tube unit (ETU). Each ETU corresponds to a row of a 96-well plate. In an aspect, the throughput of the system is about 35 minutes to the completion of the first ETU with subsequent ETUs completed at about 2 minute intervals.

20 [00103] In order to meet the throughput requirements, the instrument can process ETUs in a parallel manner. Each ETU passes through 10 steps before the processing is complete. These steps are grouped with similar steps to create six processing modules, identified by the station letters. The ETUs are moved between the six stations by a six-axis robot at about two minute intervals. Because of incubation times, some steps require the ETU to remain at the station for
25 more than about two minutes. In this case, additional locations are supplied in the station to accommodate a first-in-first-out process.

[00104] The PAS can include several components, such as: 1) an ETU transport mechanism; 2) an ETU and ETU gripper; 3) a magnet station for attracting paramagnetic beads; and 4) a pipettor station that transfers concentrated nucleic acid from ETU to plate. The PAS can
30 produce up to ten 96-well plates of extracted DNA from liquid based cytology samples in less than 5 hours for subsequent analysis in an instrument designed to run the method for determining

of the presence of the target nucleic acid molecules. The PAS is designed to address some of the current challenges of extracting DNA from liquid based cytology testing including volume of sample required (4 mL), limited automation and the low throughput of manual sample conversion protocol.

5 Target Nucleic Acid Molecules

[00105] The target nucleic acid molecules include, without limitation, nucleic acid molecules found in specimens or cultures (*e.g.*, cellular, microbiological and viral cultures) including biological and environmental samples. The target nucleic acid molecules may be found in biological samples from an animal, including a human, fluid, solid (*e.g.*, stool) or tissue,
10 as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Target nucleic acid molecules may be found in environmental samples and include environmental material such as surface matter, soil, water and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items.

15 [00106] The target nucleic acid molecules found in biological samples include, but not limited to cervical samples (*e.g.*, a sample obtained from a cervical swab) or cervical cell samples, adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, urine and semen. The target nucleic acid molecules may be from other viral, bacteria, mycobacteria or plasmodia, for example cytomegalovirus (CMV), herpes, HIV,
20 H1N1, chlamydia, gonorrhea, *Trichomonas vaginalis*, *Staphylococcus aureus*, tuberculosis, SARS-associated coronavirus or influenza. In an aspect the target nucleic acid molecules are 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to nucleic acid molecules associated with any one of cervical samples (*e.g.*, a sample obtained from a cervical swab) or cervical cell samples, adenoid cells, anal epithelial cells,
25 blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, urine and semen, other viral, bacteria, mycobacteria or plasmodia, for example cytomegalovirus (CMV), herpes, HIV, H1N1, chlamydia, gonorrhea, *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis*, *Staphylococcus aureus*, tuberculosis, SARS-associated coronavirus or influenza.

30 [00107] In one aspect, the target nucleic acid molecules are human papillomavirus (HPV) and include genetic variants of HPV. A variant includes polymorphisms, mutants, derivatives,

modified, altered, or the like forms of the target nucleic acid. In one aspect, the target nucleic acid is an HPV nucleic acid. In another aspect, the HPV nucleic acid is HPV DNA of a high risk HPV type. In another aspect, the HPV nucleic acid is HPV RNA of a high risk HPV type. In another aspect the target nucleic acids are any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or any one of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89.

[00108] In another aspect, the target nucleic acid molecule is 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to nucleic acid molecules associated with any one of HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, or HPV RNA of a high risk HPV type. In another aspect the target nucleic acids are 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to nucleic acid molecules associated with any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or any one of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89.

[00109] As noted previously, the target nucleic acid molecule may be DNA or RNA. When the target nucleic acid molecule is DNA, the probe is preferably RNA and when the target nucleic acid is RNA, the probe is preferably DNA. However, a DNA probe can be used with DNA target nucleic acid molecule and an RNA probe can be used with RNA target nucleic acid molecule. Also as indicated previously, the target nucleic acid molecule may determine the collection medium used.

Denaturation

[00110] After the sample is collected in a detergent-based collection medium as described above, the sample may be treated with a denaturation reagent to render the target nucleic acid molecule accessible to hybridization. In one aspect, the sample is denatured with an alkaline solution. Any alkali that can bring a solution pH to about pH 12, about pH 13, or about pH 14 may be used. Additionally, any alkali that can bring a solution pH to a range of about pH 12 to about pH 13, from about pH 12 to about pH 14, and from about pH 13 to about pH 14 can be used. Suitable concentrations of alkali include from about 1.0 N to about 2.0 N, from about 1.25 N to about 1.75 N, and from about 1.25 N to about 1.5 N, and about 1.5 N as well as any number within the recited ranges. Without being limited, suitable alkali include NaOH and KOH.

[00111] In one example, the sample suspended in a detergent-based collection medium can be treated with about one-half volume of 1.75 N NaOH solution. For example, in certain aspects approximately a 50 μ l aliquot is removed from a sample suspended in a detergent-based collection medium and approximately 25 μ l of 1.75 N NaOH solution is added to the 50 μ l aliquot sample. The sample treated with the denaturation reagent can be mixed by hand mixing or mechanical shaking at about 800 rpm, about 900 rpm, about 1000 rpm, between about 600 and about 1000 rpm, or between about 600 and 1200 rpm. In an aspect, the pH of the sample after addition of denaturation reagent can be about 14. In another aspect, the pH can be about pH 12 or pH 13. Such basic pH will both nick and denature a majority of the nucleic acid in the specimen. In addition, alkaline treatment can disrupt interactions between peptides and nucleic acids to improve accessibility of the target nucleic acid and degrade protein.

[00112] Alkaline treatment of protein effectively homogenizes the specimen to ensure reproducibility of analysis results for a given sample. It can also reduce the viscosity of the sample to increase kinetics, homogenize the sample, and reduce background by destroying any endogenous single stranded RNA nucleic acids, DNA-RNA hybrids or RNA-RNA hybrids in the sample. It also helps inactivate enzymes such as RNases and DNases that may be present in the sample. One skilled in that art would appreciate that if RNA is the target nucleic acid (as opposed to DNA), different reagents may be preferable including, but not limited to phenol extraction and TCA/acetone precipitation, and guanidinium thiocyanate-phenol-chloroform extraction.

[00113] Other methods of denaturation may be employed such as utilizing a heating step, for example, heating the sample to about 95°C to separate the strands of nucleic acid. Enzymes such as helicase may be used as well. The oil may be silicone oil. In one embodiment, an oil or oil-type substance is added to the sample prior to heating. The oil may have a viscosity of about 0.5 Cst to about 20 Cst, about 1.0 Cst to about 10 Cst, or about 2.0 Cst to about 5 Cst. In an aspect, the volume is about 5 Cst. In an aspect about 10 μ l to about 45 μ l of the above silicone oil is added to 1 mL or more of collection media and evaluated on an automated platform. One advantage of adding an oil is that the sample is heated more evenly.

[00114] In one aspect, 1.5 N to 2.0 N NaOH is added to the sample and heated. In another aspect, 1.75 N NaOH is added to the sample and heated. The sample with denaturation reagent may be heated to about 60°C to about 80°C for about 30 minutes, to about 65°C to about 75°C for

about 30 minutes, to about 67°C to about 70°C for about 30 minutes, or to about 70°C for about 30 minutes, or any number within the recited ranges. In another aspect, the sample with denaturation reagent is heated to about 60°C to about 80°C for about 20 to about 40 minutes, or to about 65°C to about 75°C for about 20 to about 40 minutes, to about 67°C to about 70°C for
5 about 20 to about 40 minutes, or to about 70°C for about 30 minutes, or any number within the recited ranges. A goal of the described time and temperature conditions is to provide for maximal denaturation of the sample in a minimum amount of time, while leaving the target nucleic acid in a suitable condition for carrying out the remaining steps of hybridization, capture, washing, and detection. Therefore, the sample may be heated in denaturation reagent for about 5
10 to about 120 minutes, about 10 to about 60 minutes, about 20 minutes to about 40 minutes, about 30 minutes, or any number within the recited ranges. It will be readily understood by one of ordinary skill in the art that longer periods of incubation at lower temperatures, or shorter periods of incubation at higher temperatures, may be balanced to provide a similar effect to the conditions described herein.

15 **Hybridization and Binding of Probes**

[00115] After the sample containing the nucleic acid is denatured, it is contacted with one or more polynucleotide probes under a condition sufficient for the one or more polynucleotide probes to hybridize to the target nucleic acid in the sample to form a double-stranded nucleic acid hybrid. The probe can be full length, truncated, or synthetic DNA or full length, truncated,
20 or synthetic RNA. If the target nucleic acid is DNA, then the probe may be RNA and if the target nucleic acid is RNA, then the probe may be DNA. Preferably, the one or more polynucleotide probes are diluted in a probe diluent that also can act as a neutralizing hybridization buffer (to neutralize the basic denaturation reagent).

[00116] The probe diluent used for DNA or RNA probes will differ due to the different
25 requirements necessary for DNA versus RNA stability. For example, if the probes are RNA, it is preferable to neutralize the sample first and then add the probe or alternatively, add the RNA probe and neutralizing agent (probe diluent) to the sample at the same time as NaOH can destroy RNA. The probe diluent can be used to dissolve and dilute the probe and also help restore the sample to about a neutral pH, *e.g.*, about pH 6 to about pH 9, to provide a more favorable
30 environment for hybridization. Sufficient volume of probe diluent, preferably one-half volume of the sample, may be used to neutralize the base-treated sample.

[00117] In an aspect, the probe diluent comprises a buffer, polyacrylic acid, NaOH and sodium azide. The probe diluent may comprise acetic acid. In one aspect, the probe diluent comprises 2.2 M BES (N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 2.6% polyacrylic acid (PAA), 0.7 N NaOH and 0.09% sodium azide. The probe diluent may contain from about 5 1.2 M to about 2.6 M BES, from about 1.5 M to about 2.5 M BES; from about 1.75 M to about 2.25 M BES; from about 2 M to 2.4 M BES, or about 2.2 M BES, as well as any number within the recited ranges. In one aspect the probe diluent may contain from about 2% to about 3.0% PAA or, as well as any number within the recited ranges. In another aspect, the PAA concentration is from about 2.2% to about 2.7%. In yet another aspect, the PAA concentration is 10 about 2.6%. In a further aspect the probe diluent may contain from about 0.6 N to about 0.8 N NaOH, for example, about 0.7 N NaOH. The concentration of NaOH generally increases as the amount of BES increases.

[00118] The probe diluent has a viscosity that permits accurate dispensing by automatic pipetting techniques. In other words, the viscosity of the probe diluent is adjusted so that the 15 desired volume can be accurately and automatically pipetted. If the viscosity is too low, the probe diluent cannot form a stable drop. On the other hand, if the viscosity is too high, the probe diluent drop will be too large. When such a drop enters the sample tube, it may cause significant disturbance of the contents already in the sample tube (*e.g.*, by splashing against the walls of the sample tube).

[00119] For full length probes, a heated alkaline solution may be added to the sample, then probe diluent may be added to the sample at room temperature, and then the sample may be reheated. Such a process can inhibit secondary structure from forming. Antibodies tend to 20 irreversibly bind to structures with secondary structure. When using non-full length probes such as truncated or synthetic probes, heating the solutions or sample may not be necessary because secondary structures issues are not present. In an aspect, the sample is not heated when used 25 with truncated or synthetic probes.

[00120] After treatment with the denaturation reagent, an aliquot of neutralization buffer, in an aspect the probe diluent described, in which the one or more probes are dissolved, can be added to the sample under appropriate conditions to allow hybridization or binding of the probe 30 and the target nucleic acid to occur. The neutralization buffer may contain a single buffering salt. In an aspect, the neutralization buffer does not contain more than a single buffering salt.

The hybridization condition is sufficient to allow the one or more polynucleotide probes to anneal to a corresponding complementary nucleic acid sequence, if present, in the sample to form a double-stranded nucleic acid hybrid.

[00121] Hybridization conditions suitable for the particular probes and diluents described

5 herein are employed. For example, the probes and sample nucleic acids can be incubated for a hybridization time, preferably at least about 5 to about 30 minutes, about 5 to about 20 minutes, or from about 7 to about 15 minutes, or about 10 minutes, as well as any number within the recited ranges sufficient to allow the one or more polynucleotide probes to anneal to a corresponding complementary nucleic acid sequence. The hybridization condition can include a
10 hybridization temperature of at least about 65°C, about 68.5°C, and about 67°C to about 70°C, as well as any number within the recited ranges. For a given target nucleic acid and a given probe, one of ordinary skill in the art can readily determine desired hybridization conditions by routine experimentation. One of ordinary skill in the art will further appreciate that the time and temperature of hybridization must be optimized, one with respect to the other. Thus, higher
15 hybridization temperatures may be carried out for shorter periods of time and vice versa.

Without being limited, stringent hybridization conditions may be controlled by increasing the temperature, increasing the ionic conditions to above 0.5M (for example, NaCl), or reducing the concentration of PAA. As a non-limiting example, stringent hybridization conditions may include performing a hybridization reaction at elevated temperatures, such as of at least about
20 65°C, at least about 68.5°C, between about 67°C to about 70°C, and between about 69°C to about 70°C. Stringent hybridization conditions may also include elevated temperatures, such as of at least about 65°C, at least about 68.5°C, and between about 67°C to about 70°C.

[00122] In a non-limiting aspect, the probe is capable of hybridizing or binding to nucleic

25 acid molecules 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to nucleic acid molecules associated with HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, or HPV RNA of a high risk HPV type, or any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or any one of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the probe is complementary to HPV, genetic
30 variants of HPV, HPV DNA of a high risk HPV type, HPV RNA of a high risk HPV type, or any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or

any one of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the probe is a strategically-truncated probe 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, or SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, or complements thereof.

[00123] In one aspect, the sample is suspended in detergent-based collection medium, the target nucleic acid is denatured with a denaturation reagent, and hybridized to nucleic acid probes suspended in a neutralizing buffer. In another aspect the neutralizing buffer is the probe diluent of the present invention. The probe diluent can comprises 2.2 M BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 2.6% polyacrylic acid, 0.7 N NaOH and 0.09% sodium azide.

Capture

[00124] After the probes are allowed to hybridize to the target nucleic acid molecule and to form a double-stranded nucleic acid hybrid, the hybrid is captured by a molecule that is specific for the double-stranded nucleic acid hybrid. Molecules specific for the double stranded nucleic acid hybrids include, but are not limited to, monoclonal antibodies, polyclonal antibodies, proteins such as but not limited to RNase H, nucleic acids including but not limited to aptamers, or sequence specific nucleic acids. Aptamers are short stretches of random sequences that are successively selected from a library of sequences by hybridizing to a target, amplifying the hybridized aptamers, and repeating the selection process. In one aspect the molecule specific for the double stranded nucleic acid hybrid is captured by an antibody, known as an anti-hybrid antibody.

[00125] In one aspect, a first anti-hybrid antibody is immobilized onto a support using techniques that are standard in the art. Examples of suitable supports include covalent linkages or adsorption, for example, protein-protein interactions, protein-G beads, biotin-streptavidin interaction, EDAC to link to a carboxyl or tosyl group, etc., or hybridization directly onto the solid support using, for example, sequence specific nucleic acids in an affinity column.

[00126] Supports include but are not limited to beads, magnetic beads, which as indicated previously include paramagnetic, diamagnetic, ferromagnetic, ferromagnetic, and diamagnetic

beads, columns, plates, filter paper, polydimethylsiloxane (PDMS), and dipsticks. Any support can be used as long as it allows extraction of the liquid phase and provides the ability to separate out bound and unbound antibodies. Magnetic beads are particularly useful in that they can be left in the solution and the liquid phase can be extracted or decanted, if a magnetic field is applied to immobilize the beads. Beads that are small and have a high surface area are preferable, such as beads about 1 μm in diameter. Other beads that employ charge switching or silica capture (as opposed to magnetic fields) may be used as well.

[00127] The hybrids are incubated with the anti-hybrid antibody attached to the support for a sufficient amount of time to allow capture of the double-stranded nucleic acid hybrids by the immobilized anti-hybrid antibodies. In an aspect, the support is a bead.

[00128] The anti-hybrid antibody may be monoclonal or polyclonal. In one aspect the antibody is monoclonal. In one aspect, the antibody is coupled to support by an 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDAC) linker. In one aspect, the support is a polystyrene bead. In an aspect, the support or bead coupled to the antibody is diluted in a bead dilution buffer. The bead dilution buffer is helpful in minimizing protein denaturation on the bead. One example of a bead dilution buffer comprises 6% casein, 100 mM Tris-HCl, 300 mM NaCl, and 0.05% sodium azide.

[00129] In an aspect, the beads coated with the anti-hybrid antibody are incubated with the sample at about 67°C to about 70°C for about 30 minutes. In another aspect, the beads and sample are incubated at about 68°C to about 69°C for about 30 minutes. In yet another aspect, the beads and sample are incubated at about 68.5°C for 30 minutes. The incubation time can range from about 5 minutes to about 60 minutes, from about 15 minutes to about 45 minutes, from about 20 minutes to about 40 minutes, or any number within the recited ranges, and is generally inversely proportional to the temperature. It will be understood by those skilled in the art that the incubation time, temperature and/or shaking conditions can be varied to achieve alternative capture kinetics as desired.

[00130] Following capture of the target nucleic acid/probe hybrid as described above, the captured hybrid may be separated from the rest of the sample by washing away of non-captured nucleic acids.

Conjugation

[00131] Another step in the method can involve providing a second antibody that is also specific for double stranded nucleic acids hybrids or alternatively is specific for the first antibody. The second antibody may be detectably labeled, either directly or indirectly, and may be a monoclonal or polyclonal antibody. In an aspect, the second antibody is monoclonal. In
5 another aspect, the second antibody is directly labeled with a detectable marker and is monoclonal. The second antibody is used to detect the presence of double-stranded nucleic acid hybrids. In one aspect, the second antibody has a label that must react with a substrate to provide a signal that can be detected. The second antibody may be dissolved in a suitable buffer. In one aspect the buffer comprises 100 mM TrisHCl, pH 7.4, 0.5 M NaCl, 0.1 mM ZnCl₂, 1.0 mM
10 MgCl₂, 0.25% Tween 20, 0.2 mg/ml RNase A, 4% hydroxypropyl-b-cyclodextrin (cyclodextrin), 30% bead dilution buffer as discussed previously, 0.05% goat IgG, 0.09% sodium azide. In an aspect, the conjugation reaction takes place at room temperature. In another aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C. In an aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C, between 35°C and
15 about 40°C, between 40°C and about 50°C for between about 20 minutes and 40 minutes. In an aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C for between about 20 minutes and 40 minutes. In another aspect the conjugation reaction takes place at about 45°C for about 30 minutes.

[00132] It will be understood by those skilled in the art that any detectable label such as,
20 but not limited to, an enzyme, radioactive molecule, fluorescent molecule, or metal particle such as gold particle can be used. In certain aspects, the detectable label is alkaline phosphatase. Methods of conjugating a label to an antibody are known. For example, an antibody can be reduced with dithiothreitol (DTT) to yield monovalent antibody fragments. The reduced antibody can then be directly conjugated to maleinated alkaline phosphatase by the methods of
25 Ishikawa *et al.*, J. Immunoassay 4:209-237 (1983) and Means *et al.*, Chem. 1: 2-12 (1990), the contents of each of which are incorporated herein by reference in its entirety, and the resulting conjugate can be purified by HPLC. The conjugate may also be purified using any type of size-exclusion chromatography. One benefit of purification is that the conjugates of one protein to one antibody can be separated from those conjugates with other ratios of protein to antibody.

[00133] In another aspect, the double-stranded nucleic acid hybrids can be detected with a second anti-hybrid antibody that is not directly labeled. For example, the second antibody can be a mouse immunoglobulin that is detected by a labeled goat anti-mouse antibody.

Wash

5 [00134] Following conjugation with the second antibody, the sample is washed with a based wash buffer. The wash buffer may contain one or more detergents or may be free of a detergent. If the wash buffer contains a detergent, the detergent may be an ionic or a non-ionic detergent. One example of a non-ionic detergent is Triton-X. The detergent may be present in the wash buffer at a concentration of about 0.05% to about 1.5%, or from about 0.075% to about
10 1.0%, or from about 0.1% to about 0.75%, or about 0.5% or any number within the recited ranges. One example of a suitable wash buffer comprises 40 mM Tris, pH 8.2, 100 mM NaCl, 0.5% Triton-X 100 and 0.05% sodium azide.

[00135] In an aspect, the wash buffer contains 0.5 mM to about 2.0 mM Tris and 0.02-0.10% sodium azide at a pH between about 7.5 to about 8.5. In another aspect, the wash buffer
15 comprises, consists essentially of, or consists of about 0.5 mM to about 2.0 mM Tris and 0.02-0.10% sodium azide at a pH between about 7.5 to about 8.5. In yet another aspect, the wash buffer comprises, consists essentially of, or consists of about 1.0 mM Tris and about 0.09% sodium azide. In an aspect, the wash buffer has a pH of between about 7.6 to about 8.4.

[00136] The sample may be washed with the wash buffer from one to ten times, or from
20 three to seven times, or from four to six times, four times, or five times, or any number within the recited ranges. In an aspect, the sample is washed at least four times with two different wash buffers. In another aspect, the sample is washed at least four times with three washes taking place with one buffer and another wash step taking place with a different buffer. The sample may also be washed with a single wash buffer or with multiple wash buffers. Each wash may
25 use the same wash buffer or a different wash buffer. For example, a detergent-containing wash buffer may be used for one wash while a detergent-free wash buffer may be used for another wash. In an aspect, one of the wash buffers does not include Triton.

Detection

[00137] The label present on the second, or third, or more, antibody is detected to thus
30 indicate the presence of the target nucleic acid molecule. Methods for detecting various labels are known in the art. For example, colorimetry, radioactive, surface plasmon resonance, or

chemiluminescence methods are described by *e.g.*, Coutlee *et al.*, J. Clin. Microbiol. 27:1002-1007 (1989), the contents of which are incorporated herein by reference in its entirety.

[00138] For example, a bound alkaline phosphatase conjugate can be detected by chemiluminescence with a reagent such as a LUMI-PHOS 530 reagent (Lumigen, Detroit, MI) or DR2 (Applied Biosystems, Foster City, CA) using a detector such as an E/LUMINA luminometer (Source Scientific Systems, Inc., Garden Grove, CA), an OPTOCOMP I Luminometer (MGM Instruments, Hamden, CT), or the like, such as a Veritas Microplate Luminometer by Turner Biosystems. In an aspect, a fluorometer may be used to detect the conjugate. Multiple detection techniques can also be used in sequence or in parallel. For example, the conjugate may be detected by chemiluminescence and fluorescence. In another aspect, the conjugate can be detected by chemiluminescence.

[00139] Detectors using different detection techniques for the conjugate may be reversible or irreversibly attached, for example in a modular fashion, to a machine that is capable of performing the method for determining the presence of a target nucleic acid molecule in a sample.

[00140] As described herein, detection of the label on the second antibody is indicative of the presence of one or more of the target nucleic acid molecules in the sample that are complementary to the one or more probes. Following washing, the sample is suspended in a detection buffer that for example, contains the substrate for the label on the second antibody.

[00141] In one aspect, the sample is comprised of cervical cells. The method for determining the presence of a target nucleic acid molecule in a sample of cervical cells comprises suspending the sample in a detergent-based collection medium and mixing by hand mixing. In another aspect the mixing is mechanical. An approximately 50 µl aliquot of the sample is removed and mixed with about 25 µl of a denaturation reagent. The sample is mixed by hand mixing or mechanical shaking at between about 600 to about 1200 rpm for about 30 to about 60 seconds and heated at about 70°C for about 30 minutes. High risk HPV RNA probes are prepared in a diluent and diluted to about 375 ng/ml. About 40 µl of diluted probe is added to the sample on a 70°C heating block. The samples are further incubated at approximately 68.5°C with shaking at about 1150 rpm for about 30 minutes. The supernatant can be removed by a dropper bottle or other low tech device. About 35 µl of the detection reagent is added to the sample. The detection reagent contains a second antibody that is labeled. The second antibody

is specific for double-stranded nucleic acid hybrids. The sample containing the detection reagent is incubated at about 45°C for about 30 minutes, placed on a magnetic rack for about 30 seconds to 3 minutes and the supernatant is decanted. In another aspect the sample containing the detection reagent is incubated at room temperature. The sample is then washed with wash buffer
5 about four or five times.

Anti-hybrid Antibodies

[00142] The double-stranded nucleic acid hybrids formed in accordance with the present invention can be captured and detected using antibodies that are specific to double-stranded nucleic acid hybrids. The antibody is specific to double-stranded hybrids, such as but not limited
10 to RNA-DNA; DNA-DNA; RNA-RNA; and mimics thereof, where mimics refer to molecules that behave similarly to RNA-DNA, DNA-DNA, or RNA-RNA hybrids. The anti-double-stranded nucleic acid hybrid antibody, *i.e.*, the anti-hybrid antibody that is utilized will depend on the type of double-stranded nucleic acid hybrid formed. In one aspect, the anti-hybrid antibody is immunospecific to RNA-DNA hybrids.

15 [00143] It will be understood by those skilled in the art that either polyclonal or monoclonal anti-hybrid antibodies can be used and/or coupled to beads and/or immobilized on a support in the present assay as described below. Monoclonal antibody prepared using standard techniques can be used in place of the polyclonal antibodies. Monoclonal antibodies may be produced by methods that are standard in the art. In an aspect, the antibodies used for capture
20 and detection of the target nucleic acid are monoclonal antibodies. In an aspect, monoclonal antibodies support high stringency incubation temperatures during the capture step. Without being limited, the high stringency incubation temperatures during the capture step may be between about 65° to about 75°C or between about 68° to about 75°C. The first and second antibodies may be the same for capture and detection (*i.e.*, produced by the same hybrid
25 myeloma cell line) or may be different and produced by different hybrid myeloma cell lines. In one aspect, the first and second monoclonal antibodies used for capture and/or detection are the same and are specific for RNA-DNA hybrids. Also included are immunofragments or derivatives of antibodies specific for double-stranded hybrids, where such fragments or derivatives contain binding regions of the antibody.

30 [00144] For example, a monoclonal anti-RNA-DNA hybrid antibody derived from myeloma cells fused to spleen cells that are immunized with an RNA-DNA hybrid can be used.

The hybrid-specific antibody can be purified by affinity purification against RNA-DNA hybrids immobilized on a solid support, for example as described in Kitawaga *et al.*, Mol. Immunology, 19:413 (1982); and U. S. Patent No. 4,732, 847, the contents of each of which are incorporated herein by reference in their entirety.

5 **[00145]** Other suitable methods of producing or isolating antibodies, including human or artificial antibodies, can be used, including, for example, methods that select recombinant antibody (*e.g.*, single chain F_v or F_{ab}, or other fragments thereof) from a library, or which rely upon immunization of transgenic animals (*e.g.*, mice) capable of producing a repertoire of human antibodies (see, *e.g.*, Jakobovits *et al.*, Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits *et al.*, Nature, 362: 255 (1993); and U.S. Pat. No. 5,545,806 and U.S. Pat. No. 5,545, 807, the contents of each of which are incorporated herein by reference in their entirety).

10 **[00146]** In one aspect, the target nucleic acid to be detected is DNA (*e.g.*, HPV genomic DNA or cDNA) or RNA (*e.g.*, mRNA, ribosomal RNA, nuclear RNA, transfer RNA, viral RNA, heterogeneous nuclear RNA), wherein the one or more polynucleotide probes are
15 polyribonucleotides or polydeoxyribonucleotides, respectively. In a preferred aspect, the double-stranded nucleic acid hybrids are DNA-RNA hybrids formed by hybridization of target DNA and probe RNA, and can be detected using an antibody that is immunospecific to RNA-DNA hybrids.

20 **[00147]** In an aspect of the present invention, a monoclonal anti-RNA-DNA hybrid antibody derived from a hybridoma cell line is used. Such hybridoma cell lines are described in U.S. Pat. No. 4,865,980, U.S. Pat. No. 4,732,847, and U.S. Pat. No. 4,743,535, the contents of each of which are incorporated herein by reference in their entirety. Hybrid-specific monoclonal antibodies may be prepared using techniques that are standard in the art. The hybrid-specific monoclonal antibody may be used for both capturing and detecting the target nucleic acid.

25 **[00148]** While any vertebrate may be used for the preparation of polyclonal anti-RNA-DNA hybrid antibodies, goats or rabbits are preferred. Preferably, a goat or rabbit is immunized with a synthetic poly(A)-poly(dT) hybrid by injecting the hybrid into the animal in accordance with conventional injection procedures. Polyclonal antibodies may be collected and purified from the blood of the animal with antibodies specific for the species of the immunized animal in
30 accordance with well-known antibody isolation techniques. For the production of monoclonal antibodies, the spleen can be removed from the animal after a sufficient amount of time, and

splenocytes can be fused with the appropriate myeloma cells to produce hybridomas.

Hybridomas can then be screened for the ability to secrete the anti-hybrid antibody. Selected hybridomas may then be used for injection into the peritoneal cavity of a second animal for production of ascites fluid, which may be extracted and used as an enriched source of the desired monoclonal antibodies incorporated herein by reference.

Polynucleotide Probes

[00149] The polynucleotide probes are designed to hybridize or bind with the target nucleic acid molecules. In another aspect, the polynucleotide probes are designed to bind to target nucleic acid molecules. In one aspect, the probes are capable of hybridizing or binding to HPV and HPV high risk variants. In an additional aspect, the polynucleotide probes are specific for HPV and HPV high risk variants. High risk (HR) nucleic acid probes can include probes for HPV high risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82. In another aspect, High Risk nucleic acid probes can include probes for HPV high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82. In other aspects the RNA or DNA probes are fragments. In an aspect, the probes are about 6 to about 8 kilobases in length, about 7.5 kilobases, and may be produced using a plasmid template using a BLUESCRIPT vector. However, other plasmids, vectors and methods are known in the art and could also be used to produce the RNA probes described herein.

[00150] The probes may vary in amount from about 7.5 ng to about 60 ng per HPV type per assay, or from about 20 ng to about 45 ng per HPV type per assay, or about 30 ng of probe for each HPV type per assay is used. Thus, in one aspect the HR probes consist of or consist essentially of one or more probes for HPV high risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89 wherein about 30 ng of each probe is used per assay for detection of the target nucleic acid molecule.

[00151] In another aspect, a combination or set of nucleic acid molecules is targeted. For example, a set of target nucleic acid molecules can include high risk HPV types 16, 18, and 45. In an aspect, the set of nucleic acid molecules to be targeted include only high risk HPV types 16, 18, and 45. Further, a set of target nucleic acid molecules can comprise, consist essentially of, or consist of polynucleotide probes hybridize with or are specific for high risk HPV types 16, 18, and 45 and may be used with any of the methods disclosed herein.

[00152] The RNA probes may be short synthetic RNA probes that specifically bind only to the target nucleic acid molecule. Examples are described in U.S. Pat. Appl. No. 12/426,076, filed on April 17, 2009, the contents of which are incorporated herein by reference in its entirety.

[00153] In certain aspects a probe mixture comprising multiple sets of probes is used to simultaneously screen for any one of a mixture of desired target nucleic acids. For example, it may be desirable to screen a biological sample for the presence of any HR HPV type. In such a situation, a probe set 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, or SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, SEQ ID NO: 117 may be used. For example, a probe mixture can be designed to provide a probe set for every HR HPV so one test can be run to identify whether the sample had any HR HPV target nucleic acid.

Strategically truncated Probes

[00154] In an aspect, the strategically-truncated probes are capable of hybridizing, binding to, or are specific for HPV high-risk types. In another aspect, the strategically-truncated probes are capable of hybridizing, binding to, or are specific for HPV high risk types and the deleted portion exhibits high sequence identity or cross reactivity to a HPV low risk type. In another aspect, the strategically-truncated probes are truncated at both the E1 and E2 positions of the HPV sequence where identity with LR types tends to be highest (See Figures 1-9). The strategically-truncated probes may also be truncated at the E1, E2, and L1 positions of the plasmid (See Figures 1, 4, 7-8) or truncated at the E1, E2, and L2 positions of the plasmid (See Figure 9) in areas of the HPV genome showing high sequence identity with the low risk types.

[00155] In a further aspect, the total amount of deleted sequence in the one (X) or two (XX) deletions range from approximately 1.4 to 2.4 Kb. In another aspect, the deletion is from about 100 to about 200 base pairs, from about 150 to about 300 base pairs, from about 200 to about 500 base pairs, from about 500 to about 1000 base pairs, from about 1200 to about 1500 base pairs, or from about 1000 to about 2000 base pairs. In another aspect, a first deletion is from about 150 to about 300 base pairs or about 200 to about 500 base pairs and a second deletion is from about from about 1200 to about 1500 base pairs or from about 1000 to about 2000 base pairs. In another aspect, the deleted portion exhibits high sequence identity, cross

reactivity, or hybridizes to a HPV low risk type, such as to low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89.

[00156] In another aspect, the polynucleotide probes are strategically-truncated probes about 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 116, or SEQ ID NO: 117,. In one aspect, the strategically-truncated probes are fragments from about 100 to about 200 base pairs, from about 150 to about 300 base pairs, from about 200 to about 500 base pairs, from about 500 to about 1000 base pairs, from about 1200 to about 1500 base pairs, or from about 1000 to about 2000 base pairs contiguous base pairs in length of SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 116, or SEQ ID NO: 117.

[00157] In an aspect, the one or more polynucleotide probes include a probe set of one or more probes sharing at least about 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 116, or SEQ ID NO: 117. The disclosure also provides for probe sets including one or more, two or more, three or more, four or more, or five or more of the probes described herein.

[00158] In an aspect, the strategically-truncated probes are capable of reducing cross reactivity, hybridization, or binding with low risk HPV types. In another aspect, one of more strategically-truncated probes of SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103 are capable of reducing cross reactivity with one or more low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89.

[00159] In one aspect, the present disclosure provides an isolated polynucleotide at least about 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, or SEQ ID NO: 103, OR SEQ ID NO: 117, or fragments or complements thereof.

[00160] In one aspect, the disclosure provides for an isolated polynucleotide capable of hybridizing or specifically binding to HPV 26 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 87 or SEQ ID NO: 97 (26XX), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 33 comprising a sequence at least 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 88 or SEQ ID NO: 98 (33X), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 39 comprising a sequence at least 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 89 or SEQ ID NO: 99 (39XX), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 52 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 90 or SEQ ID NO: 100 (52X), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 56 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 91 or SEQ ID NO: 101 (56XX), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 58 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 92 or SEQ ID NO: 102 (58X), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 66 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 93 or SEQ ID NO: 103 (66XX), fragments, or complements thereof; an isolated polynucleotide capable of hybridizing or specifically binding to HPV 68 comprising a sequence 50% or more,

75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identical to SEQ ID NO: 94 or SEQ ID NO: 104 (68XX), fragments, or complements thereof; and an isolated polynucleotide capable of hybridizing or specifically binding to HPV 73 comprising a sequence 50% or more, 75% or more, 80% or more, 85% or more, 90% or more, 5 95% or more, 98% or more, or 100% identical to SEQ ID NO: 95 or SEQ ID NO: 105 (73XX), fragments, or complements thereof.

[00161] In another aspect, high risk HPV sequences exhibiting cross-reactivity or specificity to low risk HPV sequences are identified for target deletions. Cross-reactivity may be predicted by percent identity from sequence alignments using any known sequence identifying 10 database, such as any of the bioinformatics tools described herein. In an aspect, after the high risk HPV sequences exhibiting cross-reactivity or identity to low risk HPV sequences are identified for target deletions, a portion of the sequence exhibiting cross-reactivity or identity to low risk HPV sequences is removed. A second, third, fourth, fifth or more deletion can be made if significant cross-reactivity is present after the first deletion. In another aspect, deletions are 15 initiated using the Invitrogen GeneTailor™ Site-Directed Mutagenesis System.

[00162] Bioinformatics tools can be employed to determine the one or more polynucleotide probes. For example, Oligoarray 2.0, a software program that designs specific oligonucleotides can be utilized. Oligoarray 2.0 is described by Rouillard *et al.*, Nucleic Acids Research, 31: 3057-3062 (2003), which is incorporated herein by reference. Oligoarray 2.0 is a 20 program which combines the functionality of BLAST (Basic Local Alignment Search Tool) and Mfold (Genetics Computer Group, Madison, WI). BLAST, which implements the statistical matching theory by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264 (1990); Proc. Natl. Acad. Sci. USA 90:5873 (1993)), is a widely used program for rapidly detecting nucleotide sequences that match a given query sequence. One of ordinary skill in the art can provide a 25 database of sequences, which are to be checked against, for example HPV high risk and low risk types 1, 2, 3, 4, 5, 6, 8, 11, 13, 16, 26, 30, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, and 89. The target sequence of interest, *e.g.* HPV 18, can then be BLASTed against that database to search for any regions of identity. Melting temperature (T_m) and %GC can then be computed for one or more 30 polynucleotide probes of a specified length and compared to the parameters, after which secondary structure also can be examined. Once all parameters of interest are satisfied, cross

hybridization can be checked with the Mfold package, using the similarity determined by BLAST. The various programs can be adapted to determine the one or more polynucleotide probes meeting the desired specificity requirements. For example, the parameters of the program can be set to prepare polynucleotides of 25nt length, T_m range of 55-95°C, a GC range of 35-65%, and no secondary structure or cross-hybridization at 55°C or below.

Cross-Reactivity

[00163] The present invention also provides for assay compositions, probes, and conditions wherein cross-reactivity between HPV HR probe sets and low risk HPV types is dramatically reduced when compared to the standard FDA approved HPV assay and probe set.

10 In one aspect, the HPV HR probe set is selected from the group consisting of HPV high risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 or low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. Using the present assay with these HR HPV probes, cross-reactivity between low risk HPV types and high risk HPV probes is reduced. *See, for example*, U.S. Pat. Appl. No. 12/426,076.

15 [00164] The present invention also provides a method for determining the presence of a target nucleic acid molecule, such as HPV, in a sample in about 2 hours or less, about 2.5 hours or less, about 3 hours or less, about 3.5 hours or less, about 4 hours or less, about 5 hours or less, about 6 hours or less, about 7 hours or less, about 8 hours or less, about 12 hours or less, about 24 hours or less, in other aspects, less than about 3.5 hours for at least 10 samples using the methods discussed above. One reason why the presence of HPV or other target nucleic acid molecules can be determined in short periods of time is because the method does not amplify the target nucleic acid molecule prior to detection. Instead of target amplification, signal amplification may be used to accurately detect the presence of HPV or other target nucleic acid molecules. In an aspect, the methods of the disclosure may include a signal amplification step.

20 In an aspect, the methods of the disclosure do not include a target amplification step. In another aspect, the methods of the disclosure may include a signal amplification step and no target amplification step.

[00165] The present disclosure also provides methods and assays for detecting cancer, for example cervical cancer, by detecting the presence of a target nucleic acid molecule, such as HPV, in a sample in about 2 hours or less, about 2.5 hours or less, about 3 hours or less, about 3.5 hours or less, about 4 hours or less, about 5 hours or less, about 6 hours or less, about 7 hours

or less, about 8 hours or less, about 12 hours or less, about 24 hours or less, in other aspects, less than about 3.5 hours for at least 10 samples using the methods and assays as discussed above.

[00166] It will be understood to those skilled in the art that the present invention can be carried out on a number of platforms including, but not limited to, tubes, dipsticks, microarrays, microplates, 384 well plates, other microtiter plates and microfluidic systems. It will be understood to those skilled in the art that the present, can be automated .

[00167] Another aspect of the present invention provides a collection medium into which samples containing the target nucleic acid are collected. The collection medium provides sample stability for several days, several weeks, or several months. For example, the collection medium may provide sample stability for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, from about 1 week to about 4 weeks, from about 1 month to about 3 months, from about 3 to about 4 months, or from about 3 month to 6 months. In another aspect, the collection medium provides sample stability for at least 21 days at 33°C or at least 6 months at 20°C. In an aspect the above sample is a cervical cell sample or a human cervical cell sample. Suitable collection media are described herein. In one aspect, the collection medium comprises, consists of, or consists essentially of NP-40, deoxycholate, Tris-HCl, EDTA, NaCl, and sodium azide. In other aspects, the collection medium comprises, consists of, or consists essentially of 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25 mM EDTA, 150 mM NaCl, and 0.09% sodium azide.

[00168] Another aspect is a detergent-containing wash buffer comprising, consisting of, or consisting essentially of 40 mM Tris pH 8.2, 100 mM NaCl, 0.1% to 0.5% Triton X-100, and 0.09% sodium azide. Yet another aspect is a detergent-free wash buffer comprising, consisting of, or consisting essentially of 40 mM Tris pH 8.2, 100 mM NaCl, and 0.09% sodium azide.

High Throughput Assay

[00169] An aspect relates to a high throughput assay and apparatus capable of being practiced with any of the methods or compositions described herein. The high throughput assay is capable of accurately and rapidly processing samples a large number of samples in a short period of time.

[00170] In an aspect, the high throughput assay is capable of processing at least 300 samples in less than 3 hours, 900 samples in about 5 hours, at least 1000 samples in about 6

hours, or at least 1500 samples in about 8 hours. In another aspect, the high throughput assay is capable of processing at least 10 microtiter plates (96 well plates, for example) in about 5 hours, at least 15 microtiter plates (96 well plates, for example) in about 7 hours, or at least 20 microtiter plates (96 well plates, for example) in about 8 hours. In an aspect, the processing of samples takes place from the start of the method or assay to the completion.

Kit

[00171] Also provided is a kit for the detection of a target nucleic acid molecule in a sample, the kit comprising, consisting of or, or consisting essentially of:

- a) a collection medium;
- b) a denaturation reagent;
- c) a polynucleotide probe;
- d) a bead coated with a first anti-hybrid antibody;
- e) a detection reagent comprising a second anti-poly hybrid antibody, wherein the second antibody is detectably labeled;
- f) a wash buffer; and
- g) a second detection reagent comprising a substrate for the label on the second antibody.

[00172] The collection medium, denaturation reagent, bead, first and second antibodies, polynucleotide probes, detection reagents, and wash buffers have been previously described.

[00173] In an aspect, the kit included one or more HPV high risk probes and the deleted portion shares high sequence identity or cross reactivity to a HPV low risk type. In an aspect, the HPV high risk probe is specific for or capable of hybridizing to one or more of HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 and the deleted portion shares cross reactivity or specificity with one or more of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89. In another aspect, the polynucleotide probes are nucleic acid sequences sharing 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 100% identity to SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, or SEQ ID NO: 117.

Apparatus

[00174] An aspect relates to a high throughput apparatus capable of being practiced with any of the methods or compositions described herein. In an aspect, the compositions, methods, assays, and kits described herein are used together with the apparatus described in U.S. Patent Application No. 12/508,304, U.S. Patent Application No. 12/508,306, U.S. Patent Application No. 12/622,131, U.S. Patent Application No. 12/605,540, and 12/605,605, each of which are incorporated by reference in their entirety. The instruments described in U.S. Patent Application No. 12/508,304, U.S. Patent Application No. 12/508,306, U.S. Patent Application No. 12/622,131, U.S. Patent Application No. 12/605,540, and 12/605,605 have broad-based applications and are capable of accurately and rapidly processing samples a large number of samples in a short period of time. Without being limited, the systems and instruments described in described in U.S. Patent Application No. 12/508,304, U.S. Patent Application No. 12/508,306, U.S. Patent Application No. 12/622,131, U.S. Patent Application No. 12/605,540, and 12/605,605, can be used to detect and analyze nucleic acid molecules associated with nucleic acid molecules associated with any one of cervical samples (*e.g.*, a sample obtained from a cervical swab) or cervical cell samples, adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, urine and semen, other viral, bacteria, mycobacteria or plasmodia, for example cytomegalovirus (CMV), herpes, HIV, H1N1, chlamydia, gonorrhea, *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis*, *Staphylococcus aureus*, tuberculosis, SARS-associated coronavirus or influenza. Moreover, the systems and instruments described in described in U.S. Patent Application No. 12/508,304, U.S. Patent Application No. 12/508,306, U.S. Patent Application No. 12/622,131, U.S. Patent Application No. 12/605,540, and 12/605,605 can be used to detect and analyze nucleic acid molecules associated with HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, HPV RNA of a high risk HPV type, or any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or any one of low risk HPV types 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89.

EXAMPLES

Example 1

[00175] In order to generate probes specific for HR-HPV having a minimal risk of cross-reacting with LR-HPV, deletion plasmids of each HR-HPV genome were generated. These

strategically-truncated plasmids include the full length sequence for each HR-HPV, minus certain regions having a relatively high degree of homology with LR-HPV nucleic acids.

1. Selection of targets for deletion

[00176] Sequence alignments are performed comparing 15 HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82) with 28 LR-HPV types (HPV 1, 2, 3, 4, 5, 6, 8, 11, 13, 30, 34, 40, 42, 43, 44, 53, 54, 61, 67, 69, 70, 71, 72, 74, 81, 83, 84, and 89) to determine overall sequence similarities. The HR-HPV and LR-HPV sequences used are set forth in Table 1 below:

Table 1

High Risk HPV	GenBank Accession	SEQ ID NO
HPV 16	K02718	1
HPV 18	AY262282	2
HPV 26	X74472	3
HPV 31	JO4353	4
HPV 33	M12732	5
HPV 35	M74117	6
HPV 39	M62849	7
HPV 45	X74479	8
HPV 51	M62877	9
HPV 52	X74481	10
HPV 56	X74483	11
HPV 58	D90400	12
HPV 59	X77858	13
HPV 66	U31794	14
HPV 68	DQ080079	15
HPV 73	X94165	16

HPV 82	AB027021	17
Low Risk HPV	GenBank Accession	SEQ ID NO
HPV 1	NC_001356	18
HPV 2	NC_001352	19
HPV 3	X74462	20
HPV 4	NC_001457	21
HPV 5	NC_001531	22
HPV 6	AF092932	23
HPV 8	PPH8CG	24
HPV 11	M14119	25
HPV 13	X62843	26
HPV 30	X74474	27
HPV 34	NC_001587	28
HPV 40	X74478	29
HPV 42	M73236	30
HPV 43	AJ620205	31
HPV 44	U31788	32
HPV 53	NC_001593	33
HPV 54	HPU37488	34
HPV 55	HPU31791	35
HPV 61	HPU31793	36
HPV 62	AY395706	37
HPV 64	N/A	38
HPV 67	D21208	39

HPV 69	AB027020	40
HPV 70	HPU21941	41
HPV 71	AB040456	42
HPV 72	X94164	43

[00177] Results of this sequence alignment are shown at Fig. 30. HR sequences showing the highest percentage of identity with LR types are identified for targeted deletions. Based on this, HPV 26, 33, 39, 52, 56, 58, 66, 68, and 73 are selected as candidate plasmids for sequence removal to reduce LR cross-reactivity.

2. Site-directed mutagenesis

[00178] Based on the HR/LR sequence alignment results, the most homologous region in each HR-HPVs initially are deleted. Bluescript plasmids comprising the full-length HR-HPV sequence of interest are methylated at 37°C for one hour. The plasmid is then amplified using overlapping amplification primers. One of the amplification primers overlaps with the region of the target selected for deletion, while the other does not. The resulting amplification products thus are unmethylated linear amplicons that correspond to the target sequence, minus the sequence selected for deletion. The target sequence and the amplicons are then transfected into an *E. coli* strain, which digests the methylated target and circularizes and amplifies the unmethylated linear amplicons. Exemplary workflows for this method are demonstrated at Figures 22–29.

[00179] The sequence of the sites that have been targeted for deletion are set forth below at Table 2:

Table 2

SEQ ID NO	HPV Type [Length]	Sequence
44	HPV26 [490bp]	CCGTTGTATGCCGGCCTGTAACATAAACTTACGCCCTAGTGGAATTGA TCCAAATCAATAGAAAATTTTCTTTTAAATCTACATCCCAAAATTTAAAT TTTTGAAAAGGATCTTCCTTTGGCACAGGAGGGGCGTTACGCTGACAGGT AGTAGCAGAGTTTTTAATAAACCTATAGGCATCTTCCAACTAGCAGTGG GAGGTAAGGTTAGTCCAAAATTCCAATCCTCCAATATGGAGGCATTTCATT AAATGTATGTAAGCCATAACATCTGTTGTAAGTGTTATTTTACACAACTGA AATATAAATTGTAATTCATATTCTTCGCCATGTCTTATAAATTGTTTATAAT CAGATGGTTTAAATGGAGTGGATGCAGATGCTGCAGATAATGTACTAATG

		GTAAGGTTAGTACTGCGGGTGGTATCAACACAGGTAACAAACAATTGATT GCCCCAACAGATACCATTATTATGACCCTGTGCACGT
111	HPV26 [1514bp]	TTTTGTTTACACACAACAGTAGAGGGCACCACCTGGTGGTTTTATACATTGC ATGTTTCCTTCACGAGCTTTATAAAATATTGCACATTCATATCGTACCAGT TTCCAATAATCAATTTGATCAGTTAATTTATTACTGTCCAGTTCATAATAGT CTAGTATTTTCTCCTGGCAGCGATTTAAACGCTGGCAAAGGTTCTCCATTT TCTTTGTCCGCGTCCTCCTCCAAATCTAATCTGGACCAGGTGGTGGAAAAA AAGCTTTTCCAATTTACATCAGTCAATGCATATACAGGATTCCCATTGCTG TCAAATGGAAATGTATTTGGAAATGGTATCACTGTTACTCTACTATGTAAA TACAAAAGTGAGTTATCTTCTTGAGGATTTATATTTGAGGTAATTATTAAT GGGGGGCATGTAACTTGCAGTAGGCTTCTATGTTTTCTGTCTATACAACAG GGATTTCCATCTAAAAAGTTACGTAAATATTTATCAATATATAACCAGCAG CTATATGTAGCATCATCTAATACTGCAACTTTTGCATCTTCTAAAGGCTGC AGCCAAAAATGGCTATTTGAATTTACATATGAAATGACTGACCCTTGCTA AATTTTATAAACTCATTGCAAACTGTGACTTACCAGTATTTGGCGGTCCA TATATTACTATACAATTGTGCTTTGGGGTACCCTTTAAAACTGTTTAAAC ACTTGTAATAAAATAAAATAAGTTTACATGTTGAAACCTTAAAAATTTGGC AATTTCTTCCACGACCCGCCCTCTTCTATTTTAGAACATCTATATTGTAGC CATTGTGACATACATAGATCGTTTCTGTGCTCTTTTATAATGTCTAGTCA TGGTTGCACAGTCTTTTACATATTTTGCCTGACAATTGCTTTTTAAAAAGG CAGCTGCATTACTATCTATGTCAGCTAACTGTGCATATTTAAATGCAATTT CACTATCATCTGTTATGTCATGATCGAACGCCCATTCACCATTTTGTGATA AATCAAATGTAGCATCATCAAACTATGTTCTAATTGTGTTTGTCTGCTACTA TCCATTCTGGTGTATCTCCATATGTCTCACTTATATTGGACAACCCTGTTTT ATAAAAAATATAATGCTACTGCTGTACTTCGCAATTTTGGTGGTTCAATTAG TAATTGCGTTTCTGGCACATTTAATAACATACATAGGCAGTTTAAATTGT TGTTCTGTTTTTGCACATGTAAAGCGCACTAGCATTAGTACTATTACTCCC CAATTACATGTAAACATTGTATATGATAATATAAACAATATTGTTGTATT AATGATTTAATACTTTCTGCTACAGAGCCTGCCACACCGAATGCTGCACAC ACCCAATCTGAACAGCATGTTTTGTCACTTTTAAACACCCGTAAGTTCT GCAAACTTACACCATATACTGTTTTAAATTTACTTAACAATGCTGCTTTT ACATTACTACATTTTAATAATTCACATATT
45	HPV33 [1460 bp]	GTCTCTAATGCCATTTGTAGTTCAATTACTTGAAATGCTTTGGTCTTTGA TGCTAACAAAGAAGGCACCACCTGGTGGCATAAATGTGAAAATCCCATTT GTTTGGCTGTATACAATAAAGCACACTCCATGCGTATCAGTTTCCAATGTT CAATTTGTGATGGTAAATCAGTTTATCAGCTTCGTAAAGAGCTAGCATTT TCTCCTGCACTGCATTTAAACGTGCTGATATTCCTCCATGGTTTTCTTGT CCTCTTCTCTATTAATCTAATTTGCACCACGTCCTTGAGAAAAAGGATT TCCAATTTTCATCATTTATTGCATACACTGGGTTACCATTTTCATCAAATGG GAATGGATTTTTAAATTCAAATACTGTTAATCTACTATGTAAATATGGCCA TCTAGAGTCTGTGCCTGCATTTGTATTTGAGGTAAGAAGCAGTGGTGGACA TTTTAATTGCACTAATGCCCTATGTTTCACATCTATTGAAATTTCAATTTCCA TCTAACGCATTTCTCATGTAATCATCTATATATGTCCAACCTATTGGCGTTA CATCATCTATCATTCCTATTTTGCATCTGATAATGGCTGCAACCAAAAGT GACTTTTAGAATTTACACATGATATAACACACCCTTTTAAAACTGTATTA AACTCATTCCAAAATATGACTTTCCTGTATTTGCTGGTCCACAAATTAGCA TACAGCTTTTTTTTGGTATACCTTTTAAAACTTTTAAATGCACCTAAAAA TGCTGTAAATTCATGTTTTGATATCTTAACAACGTACTATTGGTCTCCA ATTTCTCCATCATTTGTTTTTTCACATCTACTTTGTATCCATTGTCTATTG ACATTTTACGTTTTTCTGCTTTTTTATAATGTCTACACATTATTCACAGTC CTTACTATTTTGTGTGAGTTACTTTTTAAAAATGCAGCAGCATTACTA

		TTTGAATCTGCAAGTTGTGCATAATAATATGCAATGTCACTATCGTCCGTT AACTCGTTATCATATGCCACTGTACCATTTCACCTTAAATCAAATATATTA TCATTAAAGCTATGTTGTAAAACAGTTAGTCTATCTATCCATTCAAGGTGTT GTACCTTGACATCACTAATGTTTGACATTGCTGTTCTAAACCAATACAAT GCACATGTTTGGCTCCGTAATTTTGGTGGCTCTATAACCATAACATGTTTCA GGTATTGATAATAAAATTACTCATTAGTTTGTGCTACTGTTAACCTGTTTTTGC TACACCTAAATCTAATTAACAATAATATTATTATTCCTCTATCGCAAGTTA AACATTGTAAATGAGTATACAAACTATGCTGTTTAATTAATACTTTTAAAC TTTCTGCTACTGATGGACTAATTCCATATCCTGTTATACACCAATCTGTAC AGCTTGTTTTATCACTTTTAAATG
46	HPV33 [1459 bp]	GTCTCTAATGCCATTTGTAGTTCAATTACTTGAAATGCTTTGGTCTTTGA TGCTAACAAAGAAGGCACCACCTGGTGGCATAAATGTGAAAATCCCATT GTTTGGCTGTATAAAATATTTCCCTCCATAATGGATCCGTTTCCAATTGTT AATTTGTGATGGTAAATCAGTTTTATCAGCTTCGTAAAGCTATAGTATTTT CTCCTGCACTGCATTTAAACGTGCTGATATTTCCCTCCATCGTTTTCTTGTC CTCTTCCTCTATTAAATCTAATTTGCACCACGTCTTGAGAAAAAGGATTT CCAATTTTCATCATTTATTGCATACACTGGGTACCATTTCATCAAATGG GAATGGATTTTTAAATTCAAATACTGTTAATCTACTATGTAAATATGGCCA TCTAGGGTCTGTGCCTGCATTTGTATTTGAGGTAAGAAGCAGTGGTGGACA TTTTAATTGCACTAATGCCCTATGTTTCACATCTATTGAAATTCATTTCCA TCTAACGCATTTCTCATGTAATCATCTATATATGTCCAACCTATTGGCGTTA CATCATCTATCATTCCTATTTTTGCATCTGATAATGGCTGCAACCAAAAGT GACTTTTCGAATTTACACATGATATAACACACCCTTTTAAAACTGTATTA AACTCATTCCAAAATATGACTTTCTGTATTGTGCTGGTCCACAAATTAGCA TACAGCTTTTTTTTGGTATACCTTTTAAAAACTTTTTAAATGCACCTAAAAA TGCTGTAAATCAATGTTTTGATATCTTAACAACGTACTATTGGTCTCCA ATTTCTCCATCATTTGTTTTTTTACATCTACTTTGTATCCATTGTCTATTG ACATTTTACGTTTTTCTGCTTTTTTATAATGTCTACACATTATTCCACAGTC CTTTACTATTTTTGCTTGTGAGTTACTTTTTAAAAATGCAGCAGCATTACTA TTTGAATCTGCAAGTTGTGCATAATAATATGCAATGTCACTGTCTGCCGTT AACTCGTTATCGTATGCCACTGTACCATTTCACCTTAAATCAAATATATTA TCATTAAAGCTATGTTGTAAAACAGTTAGTCTATCTATCCATTCAAGGTGTT GTACCTTGACATCACTAATGTTTGACATTGCTGTTCTAAACCAATACAAT GCACATGTTTGGCTCCGTAACCTTTGGTGGCTCTATAACCATAACATGTTTCA GGTATTGATAATAAAATTACTCATTAGTTTGTGCTACTGTTAACCTGTTTTGC TACACCTAAATCTAATTAACAATAATATTATTATTCCTCTATCGCAAGTTA AACATTGTAAATGAGTATACAAACTATGCTGTTTAATTAATACTTTTAAAC TTTCTGCTACTGATGGACTAATTCCATATCCTGTTATACACCAATCTGTAC AGCTTGTTTTATCACTTTTAAATG
112	HPV33 [1460 bp]	ACAGTTATGTATATAATACATACCTATATAATCTACTTTCCCTGTAACCAT AGTACATGTATCTTCCTCTATAATATATATTTACCCCAGTTTGTATAATCC ATTGTATTTTTTTTGTCAATTGTCATATTGCACAGTTACTGTTTCTCCTTGTTT TTTAAACATTTTGGTGGTTCACAAAGCCACACCTCTAAGCTTGTTTGTG CAATGTCCATTGGCTTGTACTATACTGTGATTTACTTAATGTCTCTAATGCC ATTTGTAGTTCAAATTACTTGAAATGCTTTGGTCTTTGATGCTAACAAAGAA GGCACCACCTGGTGGCATAAATGTGAAAATCCCATTGTTTGGCTGTATAC AATAAAGCACACTCCATGCGTATCAGTTTCCAATGTTCAATTTGTGATGGT AAATCAGTTTTATCAGCTTCGTAAAGAGCTAGCATTTTCTCCTGCACTGCA TTTAAACGTGCTGATATTTCTCCATGGTTTTCTTGCTCTCTCTCTATTT AAATCTAATTTGCACCACGTCTTGAGAAAAAGGATTTCCAATTTTCATCA TTTATTGCATACACTGGGTACCATTTTCATCAAATGGGAATGGATTTTAA AATTCAAATACTGTTAATCTACTATGTAAATATGGCCATCTAGAGTCTGTG CCTGCATTTGTATTTGAGGTAAGAAGCAGTGGTGGACATTTTAAATGCACT AATGCCCTATGTTTCACATCTATTGAAATTTCAATTTCCATCTAACGCATTTT

		TCATGTAATCATCTATATATGTCCAACCTTATTGGCGTTACATCATCTATCAT TCCTATTTTTGCATCTGATAATGGCTGCAACCAAAAGTGACTTTTAGAATT TACACATGATATAACACACCCCTTTAAAAACTGTATTAACTCATTCCAAA ATATGACTTTCCTGTATTTGCTGGTCCACAAATTAGCATACAGCTTTTTTT GGTATACCTTTTAAAAACTTTTTAAATGCACCTAAAAATGCTGTAAATTCA ATGTTTTGATATCTTAACAACGTACTATTGGTCTCCAATTCCTCCATCAT TTGTTTTTTCACATCTACTTTGTATCCATTGTCCTATTGACATTTTACGTTTT TCTGCTTTTTTATAATGTCTACACATTATTCCACAGTCCTTTACTATTTTTG CTTGTGAGTTACTTTTTAAAAATGCAGCAGCATTACTATTTGAATCTGCAA GTTGTGCATAATAATATGCAATGTCACCTATCGTCCGTTAACTCGTTATCAT ATGCCCACTGTACCATTTCACTTAAATCAAATATATTATCATTAAAGCTAT GTTGTAAAACAGTTAGTCTATCTATCCATTGAGGTGTTGTACCTTGACAT CACTAATGTTTGACATTGCTGTTCTAAACCAATACAATGCACATGTTTGGC TCCGTAATTTTGGTGGCTCT
47	HPV39 [1692 bp]	CAGTAGTGGGTACCGATCCGTCACCTGGTACTGCACATAGAGTCAGGAC AATGAATTATGTTGCCATTATAATGCACTTCCCATTTGCCACTAGTCCCAT ACCTTTCGCATCTTGAATAAACACTTCATAGTATACTTTTAGGTGCTCGTT CATATAATATATACCCCAATAGTCCACACACCCCTTCTGTTTTACCCATAT GTCTATATTATTTTTATAATATATAGCACCCCATATAATACATTTCATAGC ATTACATTTGTCCCCATCATACCACACCTCCACTGTAGTTCCTTGTTTTTA AAACATTGTTTTGGCTGTGTATGCCACAGTTCATTACTAGTGTCTTTAATG TCCACTCCTCTGTATTGTATTGAGTTTGTGCAACACTTTCTAGTGCCATCTG CAGTTCAATAGCTTGATATGCTTTACATTTTGAAATGTTTATGGTTGGCAC CACCTGGTGGTCAATAGTATGCATGCCACGTTCTCGTGCTGCATAAAATAT TGCATTTTCCATTCGCACACATTTCCAATAATTAATTTGATCATATATTGAT TACTGTCTTGTTTCATAGTATTCTAGTATTTTGTCTGTAAACACATTTAAAC GTTGTGAAAGTGTTTTTCATCATTGTCTCCTTCATCCTCGTCTGCTGCAAGT CTAATCTGCACCAAGTCTTTTCAAAAAAACATTTCCAGTTTTTATCATTGA TTGTGTACACTGGATTCTGTTTTTGGTCAAATGGAAATGCATTTAGGAAATT TAAACACTGTTAGCCTACTACGTAAATATGGCCACCTATCGTCTTCCACAG GATTGGTATTGGAGGTTATTAATAATGGTGGACATTTTCATTTGTAGTAAAC TTTTATATTTCTATCTAACTTATTGCATACCCATCTAATGCATTTCTCAT ATAATTATCGAAATATGACCAGCAGGTACCGGTTGCATCATCTAACATTGC TAGTTTTGCATCTGCAAGTGGTTCTAGCCAAAAGTGGCTGGTGGAGTTTAC ATATGAAATAACTGTGCCCTGTAAAAAATGCATAAGGCTCATACAAAAAT GTGACTTTCCTGTATTTCGCAGGTCCATATATACTATAACAGTTTTTTTTGGG AGTACCCTTTAAAAATTCTTTAATGCACATAAAAAGGATATAAATTCTAT TCCTTGATATCTTAAGAATTGACTATGGGTCTCCAGTCCCCGCCTTCATC ACATTTACTACACCTAAATTTTATCCATTGAGACATGGACATTTGCCTTTTT TGTGCTCGCTTGTAATGTTTACACATTGTTGCACAATCTTTTACATATTTTG CCTGGCAGTTACTTTTTAAAAAGGCTGCAGCATTACTGTTACAATCTGCTA ACATTGCATAATTAATGCTATGTCACTTTTCATCAGTATATTTCATTGTCAA ATGCCCATTTGTACCATGTCCGATAGGTCAAATACACTATCATCTATTCCAT GTTGTATAACAGTTAATCGTTGTATCCATTCTGGCGTATCCCCTGTTACCA CACTAATATTGGATATACCTGTGCGATACCAATATAGTGCTGCTACAGGGC TGCGCAGTTTAGGAGGCTCCAGAAGCATACAACCTTTCTGGAACATGTAAC AATGTACTTAATCCCTTTCTACAGTAACCTATTTTTTCCACATGTATATC TT
48	HPV 39 [190 bp]	ATACCACCACGATTCCAAAAATGTCTTGCAAACAGTTGTTCCCTACGTA AACAGAAGAACATACTGTCCCATACACATCTGCAGACATTTGCAATAA TCAGGATATTTACAAATGGATTGACAAATATCTAAAGGCACCTCACTTTTG GTTTCCTGCAATGCACCAAAGTCCATAGCTCCATAGCCAG

49	HPV52 [1376bp]	TAATAGAACCACCAAAAATTACGAAGTGCTACCTGTGCATTATATTGGTA TAGAACAGGTTTGTCTAATATTAGTGAGGTATATGGTACCACCCCAGAAT GGATAGAACAACAACAGTATTACAGCATAGCTTTGACAATAGCATATTC GATTTTGGAGAAATGGTGCAATGGGCATATGATCATGATATAACAGATGA TAGTGACATAGCATATAAAATATGCACAGTTAGCAGATGTAAATAGCAATG CTGCAGCATTCCATAAAAAGCAATTCGCAAGCAAAAATAGTAAAGGACTGT GCAACCATGTGTAGACATTATAAACGGGCAGAAAAGAAAACATATGAATAT TGGACAATGGATACAGTATAGATGTGATAGAATAGATGATGGTGGAGATT GGAGGCCTATAGTAAGATTTTAAAGATATCAAGACATAGAATTTACAGCC TTTTTAGACGCATTAAAAAATTTTTAAAAGGTATACCTAAAAAAATTGT TTAGTATTATATGGACCTGCAAAACACAGGAAAATCATATTTTGGAAATGAG TTTAATTAGGTTCTTAAGTGGATGTGTAATATCCTATGTAAACTCAAAAAG CCATTTTGGCTACAACCATTAAACAGATGCAAAAGTGGGTATGATAGATG ATGTAACACCTATATGTTGGACATATATAGATGATTATATGAGAAATGCA CTGGATGGAAATGATATATCAGTAGATGTAAAGCATAGAGCCTTAGTACA AATAAAATGCCCACCATTAAATTTTAAACAACAAATACAAATGCAGGAACAG ATCCTAGGTGGCCATATTTACATAGTAGATTGGTTGTGTTTCATTTCAAAA ACCCATTTCATTTGATGAAAATGGCAATCCTATATATGAAATTAACAACG AAAATTGGAAATCCTTTTTCTCAAGGACGTGGTGCAAAATTAGATTTAATAC AGGAAGAGGACAAGGAAAACGATGGAGTCGATACCGGCACGTTTAAATG CAGTGCAGGAAAAAATACTAGATCTATACGAAGCTGATAGTAATGACCTA AACGCACAAATTGAACATTGGAAATTGACTCGAATGGAATGTGTTTTGTTT TACAAAGCAAAGGAACTGGGAATAACTCATATAGGCCACCAGGTGGTGCC ACCAATGGCAGTGTCTAAGGCAAAGGCCCTGCCAAGCTATTGAACTACAAT TGGCATTGGAGGCATTAAACAAAACACAATATAGCACAGATGGATGGACA TTACAACAAACAAGTCTAGAAATGTGGCGTGCAGAACCAAAAAATACTT TAAAAAACATGGGTATACAATAACAGTGCAATACGATAATGATAAAACAA TACTATGGATTATACAACTGG
50	HPV56 [1961 bp]	ACGTGGAGGTGGTGGTGGTGGTCTTGTGGGTGTTGTATTTCGTTAACAGT TTCAACAGGGGATACGTTGTATCTACAGGTACTAGACACAGAGTCAGGAC AATAAATACTCTCATTTTCCATATGTACTTCCCATATGTTTTTACACCCAAA TTTTTTGGCCTCTTGTTCAAAGTCTGTGTAGTATGTTTTGTGGCCATCATGT ACATAATATATACCTCTATAGTCTACCCCAGAACACACTTTTTGCCACCCA CAATCTCCATTGTAATATATATATTTCCAGGCTACATATTGCATACAATTG TTTTTACTACCATCAAACCATACTTCTATATGTTGTCCCTCTTTTTTAAAGC ATTTTTTAGGTTTCAGTAAGCCATAGTTCCTCGCATGTGTCTCTTAATGTCCA CTCTTCATTGTTATATATTGTTGTACTTAATGATTCCAGTGCTATTTGCACT TCTATTGCACTACATGCTTTTGCTTTACATACTTGTAACAAGGCACCATC TGGTGGTTTAGTACAGTAATGTCATTTTCTCTTGCTTTATAGTATAGCACAT TTTCATGTGCGCACAGCTTTCCAATATTCTATATGATCTGCAATACATCTACT ATCTTTTTCAAACAGTCTAGTATTTTGTCTGGCACGCATTTAAACGTTG GGAAAGCGTCTCCATTGTTTTCTTTGTCTCGTCGTTATCCAAATTTAATCT GGACCACGTCTTGTAAGAAACATTTCCAGTTTACATTACTTAATTCATA TACAGGATTACCATTATTATCTAATGGAAATGGATTTTGAAACTGAAACAC TAACATTCTACTGTGTAAATATCGTAATTTAGCATCTAGCATAGGATTTAT ATTGGTTGTAATTAGTAATGGTGGACATTTTATTGTTACTAATTGTTTATGT TTTCTATCTAAACTTATAGGATTTCCATCTACCAAATTCCTTAAATAATCGT CTATATATTTCCAACATATTTCTGTTGCATCATCCAACAACCCAAGTTAG CATTGTCTAATGGCTGCAACCAAAAGTGGCTTTGTGAATTCACAAATGAA ATGACAGACCCTTGAAAAAAGCTTTATAAGACTCATAGCAAAGCATGTTT ACCTGTATTTGGCGGTCCACAAAGTACCAAACAGTTATGTTTAGGTGTTCC TTGTAGAAATAATTTAAAGTAACTTAGAAATGAAATGAAATCGACCCCTT GATATCTTAAAAATTGTACAATGGGTTTCCAATCACCCCTTCATCTGTTTT ACTACATATGTGCTTTATCCACTGGCACATATTCATTTGTTGCTGTTGTGCC

		CTTTTATAATGTCTACACATTATTCCACAATCCTTTACATATTTTGCCTGCA TATTGCTTTTTAAAAAGGCTTGTGCATTGCTGTCTACATCTGCTAATTGTGC ATATTGAAACGCAATTTGGCTATCATCTGTTACTTCATTATCAAATGCCCA CTGCACCATTTAGATAAATTCAAATTGACTATCCTGTAAACTGTGTTGCAA TTGTGTTTGTCTTTGTATCCATTCTGGTGTGTCTCCATACACATCACTAATA TTTGACATTGCTGTTTTATAAAAAATATAAAGCTACAGCAGGACTTCGTATT TTTGGTGGTTGAATTAACATTTGCTCCTGTGGTACATTTAATATTGAGCTTA ATGCTTTTGCAATTGTTTTTCTGTTTTTGGCCACATGTATATCTAATTAGCAT CATTACTATAACCCCCCATGTACATGTAAACATTGCATATGATAATACAT ACAGTGTGGTTTTATTATAGTTTTTAGTGCCTCGGCTAATGTTTCATTAACA CCAAATATAGCACATATCCAATCATTGCAACATGTAATCTACTATCACTTTTAAAC GTACGCACCAATTCTGAAAATGGAATACCATACACTTCTTTAAATTTATAA TATAATTTACC
113	HPV56 [1962 bp]	CTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTT GCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGAT AAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGG GCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTC AGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCA CTGATTAAGCATTGGTAAGTGTGACAGCAAGTTTACTCATATATACTTTAG ATTGATTTAAACTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTT TTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGA GCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT CTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCAGCTACCAAGCGGT GGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGG CTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTT AGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCT AATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGG GTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGGCTGAA CGGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAA CTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGG GAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG CGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGT CGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG GGGGCGGAGCCTATGGA AAAACGCCAGCAACGCGGCCTTTTACGGTTCC TGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCTGA TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCG CAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAG CGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCTTAATGC AGCTGGCACGACAGGTTTCCCGACTGAAAAGCGGGCAGTGAGCGCAACGC AATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTACACTTTAT GCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACAC AGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCTCACT AAAGGGAACAAAAGCTGGGTACCGGGCCCCCTCGAGATGGCGACGTG GCGGCCTAGTGAAAATAAGGTGTATCTACCTCCAACACCTGTTTCAAGG TTGTGGCAACGATTCTATGTAAAACGCACTAGTATATTTTATCATGCAG GCAGTTCACGATTGCTTGCCGTAGGACATCCCTATTACTCTGTGACTAAGG ACAATACCAAAACAAACATTTCCCAAAGTTAGTGATATCAATATAGGGTA TTAGGGTACGGTTGCCCGACCTAATAAGTTTGGGCTTCCAGATACTAAT

		ATTTATAATCCGGACCAGGAACGGTTAGTGTGGGCATGTGTAGGTTTGGAGGTAGGCCGCGGACAGCCTTTAGGTGCTGGGCTAAGTGGCCATCCATTGT TTAATAGGCTGGATGATACTGAAAGTTCCAATTTAGCAAATAATAATGTTA TAGAAGATAGTAGGGACAATATATCAGTTGATGGCAAGCAAACAC
51	HPV56 [210bp]	TTAGCTAATGGGTCCTGTTTTCTGTTGGTGGCTGTTCCCGTTGACATGT TATAGCTGTGCTTCTAACATATCTATATTTATCTTCTAGGCTGGTGGCCACT GGCGGGGATAACCCAATATTCCAGTCCTCCAGTAGGTTAGCATTTCATATTA TGTAATATAGCCATAACCTCTGCAGACAAAGTAATTTTGCATAATTGAAA AACAAAT
52	HPV58 [1300 bp]	TTGTGATGTTAAATCATTTTTATCAGCTTCGTATATGTCTAGGATTTTGT CCTGCACTGCACTTAAACGTGCTGATAATTCCTCCATCGTTTTCTTGTCTT CTTCCTCTATTAAGCCTAATTTGCACCACGTCCTTGAGAAAAAGGATTTCC AATTTTCATCATTTATTTTATACACTGGATTACCATTTGCATCAAATGGAA ATGGATTGTTAAATTCAAATACTGTTAGTCTACTGTGCAAATATGGCCATC GTGAATCTTTGCCGTGCATTGTATTTGAGGTAATTATTAATGGTGGACATT TTAATTGTAATAATGCCCTATGTTTTACATCTATTGAAATGTCGTTACCATC TAATGCATTTCTCATATAATCATCTATATATGTCCAGCTTATGGCTGTTACA TCATCTATCATACCTAGTTTAGCATCTGATAATGGCTGCAACCAAAAATGA CTTTTGGAATTTACATATGAAATAATGCATCCTTTTAAAAAATGTATTAAA CTCATTCCAAAATATGATTTCCCTGTATTTGCTGGGCCACACAGTAACATA CAACTTTTTTTTGGTACACCTTGTA AAAAC TGTTAAATGCAACTAAAAAT GCTGTAAATTCAATATTTTGATATCTTAAAAATTGTA CTATTGGTCTCCAA TTACCTCCATCATTTGTTTTTCACACCTACTTTGTATCCATTGTCCCATTGT CATACCACGCTTTTCTGCTCTTTTATAATGTCTGCACATAACGCCACAGTCT TTTACTATTTTGTGTTGTGCATTGCTTCTTAAAAATGCTGCTGCATTACTAT TAACATCTGCTAACTGTGCATATTTATATGCAATGTCATCTATCATCTGTAA TGTCAATTATCATATGCCCATTTGTATCATTTCACTTAAATCAAATATATCATC ATTAAGCTATGCTGTAACACTGTAAATCTATCTATCCATTCTGGTGTGTC CTTGCACATCACTTATATTTGACATTGCTGTTCTAAACCAATATAAGGCA CATGCTTGACTTCGTAATTTTGGTGGCTCGATAATCATACATGTTTCAGGA ATTGATAGTAAATTACTCATTAATTTTGGCACAGTTAATCTATTTTTGCTAC ATTTAAATCTAATTAACAATAATAATAATTCCTCTGTACACAGTTAAAC ATTGTAGGTGTGTATATACTGTGCTGTTAATTAGTACTTTTAACTTTC TGCTACGGAGGGACTTATCCATACCTGTTATACACCAATCTGTACAGCT TGTTTTATCACTTTT

53	HPV66 [1655bp]	TTTTTTAAACAGTTTTTTGGGCTCCGTGCGCCACAGTTCATCACATGTA TCACGTAATGTCCACTCTTCATTTTTATATATTGTGTTACTTATTGCTTCCA GTGCCATTTGTAATTCTATTGCACTACATGCTTTTGCTTTACACACTTGTA AGAGGGCACCATCTGGTGGTTTAGTACATTAATGTCATTTCTCTTGCTTT ATAATATAATACATATTCATGTCGTACAGCTTTCCAATAGTTTATATGATC TATAATGCATTTACTATCTTTTTCATAACAGTCTAGTATTTTGTCTGGCAC GCATCTAAACGTTGGGATAGAGTCTCCATTGTTTTCTTTGTCTCGTCGTTA TCCAAATTTAATCTGGACCATTGTCCTTTCAAAAAAACATTTCCAATTTACA TTACTCAATTCATACACAGGATTACCATTGTTATCTAATGGAAATGGATTT TCAAACTTAAACACTGAAATTCTACTGTGTAGATATCCTAATTTTGCATCT TGCATAGGATTTACATTAGTTGTAATAATGACTGGAGGACATTTTATTTGT ACTAATTGTTTATGTTTCTATCTAAACTTATGGGATTCCCATCTAATAAAT TTCTTAGATAATCATCTATATATCTCCAACACGTATCTGTTGCATCATCCA GCAAACCTAATTTGGCATTGTCTAGTGGCTGTAACCAAAAGTGGCTTTGTG AATTAACAAATGAAATGACTGACCCTTGAAAAAATTTATAAGGCTCATA GCAAAACATGATTTACCTGTATTTGGTGGTCCACACAGTACCAACAATTA TGTTTAGGCGTGCCTTGTAATAAATTTAAATAAATTTAAAAATGAAAT GAAGTCGACCCCTTGATATCGTAAAAATTGCACAATGGGTTTCCAATCACC AAGCCAAGCTTAATTCGGCTTCCCTTCATCTACTTTACTACATATATGCTTT ATCCACTGGCACATATTCATTTGCTGTTGCTGTGCCCTTTTATAATGTCTAC ACATTATTCACAATCCTTTACATATTTTGTGTCATATTACTTTTAAAAA TGCTTGTGCATTACTATCTATGTCTGCTAGTTGTGCATATAAAAAGGCAAT TTGGCTATCATCTGTTACGTCATTATCAAATGCCCACTGTACCATTTTAGA CAATTCAAATTGATTGTCTTGTAACCTGTGTGCAATTGTGTCTGTCTTTGT ATCCATTCTGGTGTTCCTCCCATACACCTCACTAATATTTGACATTGCTGTT TATAAAAATATAATGCCACAGCAGGACTTCGTAGTTTGGTGGTTGAATTA ACATTTGCTCTTGTTGGTACATTTAAAAATTGAGCTTAGCGATTTTGTAAATGT TTTTCTGTTTTTCCACATATATATCTAATTAGCATCATTACAATTACCCCC CATGAACATGTTAGGCATTGCATATGATAGTACACACATGTGGTTTTAGT ATAGTTTTTAACGCTTCTGCTAATGTTTCATTAACACCAAAATTTGCACAT ATCCAATCGTTACAACATGTACTATCGCTTTTAAATGTTTCGCACCAACTCT GTATATGGCACTCCATACACTTCTTTAAATTTAAATGTAATCTTCCTTGTA CGTTACTACTTTTA
114	HPV66 [1634bp]	CGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAA TTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCA ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTA TGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCC CCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCA GAAGTAAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCACTGCAT AATTCTTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAG TACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCT TGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAA AGTGCTCATCATTTGGAACACGTTCTTCGGGGCGAAAACTCTCAAGGATCTT ACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAATGATC TTCAGCATCTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAG GCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATA CTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTC TCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGG GTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAAATTTGTAAGCGTTAATA TTTTGTTAAAAATTCGCGTTAAATTTTTGTTAAATCAGTCTATTTTGAACCA ATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGA TAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAAGAAC GTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCC ACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAA

		AGCACTAAATCGGAACCCCTAAAGGGAGCCCCGATTTAGAGCTTGACGGG GAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAG CGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACC ACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTGCGCCATTC AGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATT ACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAA CGCCAGGGTTTTCCCAAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGC GCGTAATACGACTCACTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGG CCGCCTGTAAAAAATAGGGAACACGTTTACGGCGCCTACGTTTAAAAAA ATATACAGGCCATAGTGCAAATGTACCTCCCTGTATATATACATCATGTGT AACATCATAAGGAGACTGAGGTACAAAGGGCCAAGTACTGGGGCCTGTA GGTAAACTATATCAGGACCA
54	HPV66 [320bp]	GATTACCCAGCATATGCCATTATTATGGCCCTGTGCACGTTGCAACCA ATAAGGTTTATTAAATAATTGGGCCTCAGAGGTAATCATGGACCCACTAG GAGTAGCAACATATACAGAACTGGGAGGAGGGTCCCTGCCATTGCCACCC TTCCAATACAAATCTGTAGGAATGGCTTCCCCAACATTACCTGCCCTATTA AAGTAATGTTTGGCAAACAATTGTTCCCTGCGTAAGTAAAACCATAGA ATCCCCATAGGCATCTGCAGACATTTTGTAGATAATCAGGATATTTACATGT AGATTGTACAATGTCCAAT
115	HPV66 [320bp]	GCGTGCCCTGTAAAAATAATTTAAATAACTTAAAAATGAAATGAAGTCG ACCCCTTGATATCGTAAAAATGCAACAATGGGTTTCCAATCACCAAGCCA AGCTTAATTCGGCTTCCCTTCATCTACTTTACTACATATATGCTTTATCCAC TGGCACATATTCATTTGCTGTTGCTGTGCCCTTTTATAATGTCTACACATTA TTCCACAATCCTTTACATATTTTGTCTGCATATTACTTTTAAAAATGCTTG TGCATTACTATCTATGTCTGCTAGTTGTGCATATAAAAAGGCAATTTGGCT ATCATCTGTTACG
55	HPV68 [1930bp]	GAGTTTGTTTTTTTTGGTGCTAGGGGCACCTGCGGTGGTATGGGTGCGCG TGGTGTCTGTAGGTCGGCAACAGATTGAGTAGTGGATACTTTTCCGTCAG TGGTACTGCACATAGAGTCAGGACAATGGATTATGTTGCCATTATAATGC ACGTCCCATTTTCCACTAGTCCCATATAGTTGTGCATCCTGCATAAACCTTT CGTAATAGGTTTTTTGTTGTTTCATACATATAATATACACCCAGTAATCCA CACGCCCTTGGGTTTTACACCATGTGCTGTACTGTTTTTAAAGTAAATGT ACCCACACTACATAATGCATTGAGTTACTCTTGTCCCCATCATACCATAC TTCCACTGTAACACCATGTTTTTTTAAACATTGCTTTGGCTTTGTATGCCAT AGTTCATTACTTGTGTCCCTTAATGTCCACTCCTCTGCACTATATGCAGTTT TAGCAAGGCTCTCTAGTGCCATCTGCAGTTCAATAGCTTGATATGCTTTAG TTTTTGAAATGTTTACAGGCGGCACCACCTGGTGGTCAATATTATGCATAC CACGTTCTCGTGCTGCATAATATATTGCATTTTCCAGTCGCACACAATTCC AATAGTTAATATGGTCCTGTATACATTTACTGTCTGTTTCAATATGTTCTAA TATTTTCTCCTGTAACACATTTAAACGTTGGGAAAGTGTTCATCATTGTC TCCTTCATCCTCGTCCTGCTGCAAGTCTAATCTGCACCAAGTCTTTTCAA AAAACATTTCCAGTTTTTATCATTGATTGTATACACTGGGTTCCTGTTTTGG TCAAATGGAAATGCATTAGGAAATTTAAACACGGTTAGTCTACTATGTAA ATACGGCCACCTATTGTCTTCTACAGGGTTAGTATTGGATGTTATTAGCAT TGGTGGACACTTTATTGTATTAGGTGTCTGTGTTTTCTACTAACTAATTT GGGTACCATCTAATGCATTTCTCATGTAATTATCAAAATATGACCAGCAT GTACCTGTTGCGTCATCTAGCATGGCTATTTTGCATCTGCAAGTGGCTCT AACCAAAAGTGACTTGCTGAATTTACATATGAAATTATTGTGCCTTGTA

		AAATGTATAAGGCTCATGCAAAAATATGACTTGCCTGTATTTGGCGGCC ATGTATAACTATAACAATTCGTTTTGGCGTGCCTTTTAAAAAATCTTTAAT GCACATAAAAAATGTTATAAATCCAGTCCTTGATATCTTAAAAAATTGTACA ATTGGGCGCCAATCACCGCCTTCGTCACATTTACTGCATCTAAATTTAATC CATTGTGGCATTGTCAATTTGTCGTTTTTGCGCCCGTTTGTAAATGTCTACACA TTGTTGCACAATCTTTTACATATTTTGCTTGACAGTTGCTTTTAAAAACGC TGCAGCATTACTATTACAATCTGCTAACATAGCATATTGAAATGCTATATC ACTGTCATCTGTAACTCATTATCAAATGCCCATTTGTACCATGTCTGATAA ATCAAATACACTATCATCTATTCCATGTTGTATTATGGTTAATCTTTTTATC CATTCCGGCGTGTGCGCCACACACCTCACTAATATTAGATATTCCTGTTCTA TACCAATATAATGCTGCAACAGGGCTACGTAATTTTGGTGGCTGCAAAAG CATACAGCTGTCTGGAACATGCAACAATGTACTCAATCCTTTTCCCTACTGT TATTCTATTTTTTCCACATTTGTATCTTATTAGCATTAGTATTAATATTTCCA GTTTTTGTATCTAAACATTTGTATATGGGTATACAATGCATATTGTTAATTA GTGTTTTAAACCCTTCCGCAATGGTTGGATTACTCCAAATATTGCTGCTA CCCAGTCTGTACATGTGGTTTTATCACTTTTAAAT
56	HPV68 [450bp]	GCAAATAACTGTTCCCTACGTAAACAAAAAACATACTGTCTCCATAC ACATCTGCAGACATTTGCAAATAGTCAGGATATTTGCAAACAGATTGACA TATATCCAAAGGTACCTCGCTTTTCGTTTCTTGTAAATGTACCAAAGTCCAT AGCACCATATCCTGTATCAATCATATCGCCATCCTCAATAGGAGTATTTAC CAATTCCAATGGGGGACAGTCCCTTGTGTACATTGGTAGGCTTACAAGA TTTACCTTTGGCCCAGTGTGCGCCAATAGCAGGAACACAGCCTATAATACA CAGCTGTGTTTGTACAGTCCACTGCAACATTGTCCCTACTGTCTTTAGG ATTTTTATTAGAGGAAAACGGGGAATTTTCAGTATCATCCAGCCTATTATA TAGTGGATGCCCCACTAAGGCCAACGCCCAATGGCTGCCCCCTACCT
57	HPV73 [640 bp]	ATGTTGGCTTTGGAGCCATGGATTTTAAAGCTTTACAAGCAAATAAAA GTGATGTACCTATTGATATTTCTAACACTACCTGTAAATACCCAGATTATT TAGGCATGGCTGCTGATCCCTATGGTGATTCCATGTGGTTTTATCTTCGTA GGGAACAAATGTTTGTTCGACACTTATTTAACAGGGCTGGTGATACCGGT GATAAAATCCCAGATGACCTAATGATTAAAGGCACAGGCAATACTGCAAC ACCATCCAGTTGTGTTTTTATCCTACACCTAGTGGTTCCATGGTTTCTTCA GATGCACAGTTGTTTAATAAACCTTATTGGTTGCAAAAGGCACAGGGACA AAATAATGGTATTTGTTGGCATAATCAATTATTTTAACTGTTGTAGATAC TACTAGAAGCACTAATTTTCTGTATGTGTAGGTACACAGGCTAGTAGCTC TACTACAACGTATGCCAACTCTAATTTTAAGGAATATTTAAGACATGCAGA AGAGTTTGATTTACAGTTTGTTTTTTCAGTTATGTAAAATTAGTTTAACTACT GAGGTAATGACATATATACATTCTATGAATTCTACTATATTGGAAGAGTGG AATTTTGGTCTTACCCACCACCGTCAGGTAC

[00180] Linear probes are then generated from the plasmids. This can be accomplished in a number of ways, including but not limited to: cleaving the plasmid with, for example, a restriction endonuclease and/or amplifying a portion of the plasmid by, for example, sequence-specific polymerase chain reaction.

- 5 [00181] In one embodiment, the probes are generated by (1) linearizing the plasmid through the use of a restriction endonuclease, then (2) amplifying the probe portion of the plasmid using a polymerase and a polymerase specific promoter. For example, linearized RNA probes can be generated from the plasmids in Figs. 22–29 by first cleaving the plasmid with *XhoI*

restriction endonuclease, then transcribing the portion specific for HPV nucleic acids with T7 RNA polymerase. This is depicted at Fig. 31.

3. Test for cross-reactivity with LR-HPV

[00182] An *in vitro* transcription RNA probe set was generated from the truncated plasmids were tested according to the method described in Example 1 to the probe set to determine if cross-reactivity with LR-HPV was reduced or eliminated. A second deletion was made if significant cross-reactivity was still apparent after the first deletion. The deletion primer sequences are set forth in the following table:

Table 3

HPV	SEQ ID NO	SEQUENCE
26X	58	5' GTATTTC AATTGCCTGCCATGCCGTGTAACACTGTTTACATGTGTGTC 3'
	118	3' GCTGACGTTACGATATACATAAAGTTAACGGACGGTACGGCA 5'
26XX	60	5' GTTTCGGCCGTGTAACCAGTATGGCTTATTAAATAGTTGT 3'
	119	3' ATTCCCTGCAAACCACGGATCAAAGCCGGCACATTGGTCA 5'
33X	62	5' TTTATCTAATGTCTTACTAATTCCATAAACTTATTCCAT 3'
	63	3' ACCGAACATGATATGACACTAAATGAATTACAGAATGATT 5'
52X	64	5' GAAACACATATGGAAGGAAATTTATTTACTTGGTGAGTGT 3'
	65	3' GTGTCGACAATTTATATGGTCTTTGTGTATACCTTCCTTT 5'
58X	66	5' TAGACCATT TATTGAACATTGGAACTAATACGCATGGAG 3'
	67	3' CATTCAA AATACCTTAATCAATCTGGTAAATAACTTGTA 5'
39X	68	5' AATGCTAATAACTTACTACCGAATTATCAAACACCACCGC 3'
	69	3' TTTGTTCCTCATGATTAAAATTACGATTATTGAATGATGG 5'
39XX	70	5' GTCACCCACCTATCAATCATATCACCATCCTCAATAGGGG 3'
	71	3' ATGTTAACCCGTCCTTACCGCAGTGGGTGGATAGTTAGTA 5'
56X	72	5' CAATTTACAACCGTGGGCAACCAAGACGCCGCAGTATCC 3'
	73	3' GTCCTGAACAAATTTTCATCGTTAAATGTTGGCACCCGT 5'
56XX	74	5' ATGAATTACAATATAAATTTTGGGATGTTA ACTTACAGGA 3'

	75	3' GGAATCTGTACACCTCCTTATACTTAATGTTATATTTAAA 5'
66X	76	5' AGGAACTATTGAAGGACAACATATAGAAGTGTGGTTTGAT 3'
	77	3' TTGTGGTTGTGTGGTTAACGTCCTTGATAACTTCCTGTTG 5'
66XX	78	5' CTGAGGTGCCAGGTATTTGTTACTGTTGTGGATACTACCA 3'
	79	3' CGTTAATGTCCTTAGTTTCCGACTCCACGGTCCATAAACA 5'
68X	80	5' CTGGTACGTACGACGTCGTCTCGGAAGTATCCCAGACAGT 3'
	81	3' TACCTAACAGTAAATTACTGGACCATGCATGCTGCAGCAG 5'
68XX	82	5' AAAATGCCTGATTTCAACACCAACACAGGCCCATACCATG 3'
	83	3' TGGTACGGAGGGGATAAGG TTTTACGGACTAAAGTTGTG 5'
73X	84	5' ATTGCTAGCACAGTAGAGGTTAGATATGACTGTGAAAAGG 3'
	85	3' TCCTTATTACGTTTTTCGTGTAACGATCGTGTCTATCTCCA 5'
73XX	86	5' TTCCTCTAAACTATCATATCACCATCCTGTATAGGGGTGT 3'
	87	3' CTACAATGTATAGATATACAAAGGAGATTTGATAGTATAG 5'

[00183] Among the selected plasmids, the total amount of deleted sequence contained in the one (X) or two (XX) deletions ranged from ~1.4 to 2.4 Kb. The final plasmid constructs resulted in HPV 33X, 52X, 58X with single deletions and HPV 26XX, 39XX, 56XX, 66XX, 68XX and 73XX with double deletions. Restriction maps of the plasmids and resultant probes are set forth in FIGS 10 through 18. Exemplary deletions are shown above at Table 2.

4. Exemplary workflow for double deletion of HPV26

[00184] An example of this workflow for generating an HPV26 double strategically-truncated plasmid is depicted at FIG. 19 and 20. A pBluescript II KS(+) plasmid bearing an HPV26 sequence according to GenBank accession number X74472 was used as starting material. After methylation, the plasmid is amplified with the following pair of deletion primers:

Forward Primer (SEQ ID NO: 58):

5' GTATTTCAATTGCCTGCCATGCCGTGTAACACTGTTTACATGTGTGTC 3'

Reverse Primer (SEQ ID NO: 118):

5' ACGGCATGGCAGGCAATTGAAATACATATAGCATTG CAGTCG 3'

[00185] The result of this amplification deletes a 1510 base pair section of the plasmid, resulting in truncations of the E1 and E2 regions. This amplicon is then transfected into a strain of DH5 α TM - T1^R *E. coli* bearing an active form of *McrBC* nuclease (Invitrogen Corp., Carlsbad, California) to generate an HPV26X strategically-truncated plasmid.

5 [00186] A second amplification is then performed with the following pair of deletion primers:

Forward Primer (SEQ ID NO: 60):

5' GTTTCGGCCGTGTAACCAGTATGGCTTATTAAATAGTTGT 3'

Reverse Primer (SEQ ID NO: 119):

10 5' ACTGGTTACACGGCCGAAACTAGGCACCAAACGTCCCTTA 3'

This amplification deletes a 490 base pair section of the plasmid, resulting in truncation of the L1 region.

[00187] This amplicon was then transfected into a strain of DH5 α TM - T1^R *E. coli* bearing an active form of *McrBC* nuclease (Invitrogen Corp., Carlsbad, California). The *E. coli*
15 circularizes the amplicons to generate an HPV26XX strategically-truncated plasmid, while *McrBC* digests the methylated template.

[00188] The HPV26XX plasmid was restriction mapped to confirm the structure and size. The plasmid was digested with (1) XhoI only; (2) XhoI and NotI; (3) BamHI and SpeI; and (4) HindIII. The results are shown at Fig. 21. This plasmid has the features set forth below in Table
20 3:

Table 4

	Name	Starts At (nucleotide)	Ends At (nucleotide)	Coding Strand
<u>Coding sequences</u> <u>(7 total)</u>	Ampicillin Resistance Gene	1233	2093	Complementary
	L2	2878	4296	Complementary
	Truncated E2	4622	5521	Complementary
	E1/E2 Deletion (1510 bp deletion)	5542	5542	Not Applicable
	Truncated E1	5538	6093	Complementary

	E7	6101	6415	Complementary
	E6	6422	6860	Complementary
	L1 Deletion (490 bp deletion)	7809	7809	Not Applicable
	Truncated L1	7796	8818	Complementary
<u>Misc. Feature</u> <u>(1 total)</u>	pBluescript II KS(+)	1	2891	Not Applicable
<u>Prokaryotic Promoter</u> <u>(1 total)</u>	T7 Promoter	2855	2874	Not Applicable

Example 2: Assay Using Cervical Samples and HPV probes

[00189] A total of 324 physician collected cervical samples were collected in a detergent based collection medium and tested for the presence of high-risk HPV.

5 [00190] A 1 ml sample was vortexed to homogenize the sample and a 50 µl aliquot was removed and combined with 25 µl of denaturation reagent (1.75 N NaOH) in the assay microplate. This was shaken to mix and incubated at 70°C for 30 minutes to create single stranded DNA. To this, 40 µl of a neutralization buffer (probe diluent - 2.2M BES, 2.6% PAA, 0.7 N NaOH and 0.09% sodium azide) containing RNA probes for 16 HPV types was added to
10 create a neutral pH and incubated at 68.5°C for 10 minutes.

[00191] Following this, 10 µl of antibody conjugated paramagnetic beads (approximately 1 µm carboxylated SERADYN beads from Thermo Fisher) were added to the reaction and incubated for an additional 30 minutes at 68.5°C. The RNA probes and DNA target molecules that were complementary to each other bind and create RNA-DNA hybrids. The hybrids then
15 captured by a RNA-DNA hybrid specific antibody coated on the paramagnetic SERADYN beads.

[00192] Following incubation, the paramagnetic beads are separated from the liquid phase/supernatant by exposure to a magnetic field. The supernatant waste is removed by decanting and 35 µl of detection reagent 1 (secondary antibody conjugated enzyme comprising a
20 monoclonal anti-RNA-DNA hybrid antibody conjugated to alkaline phosphatase) is added and incubated at 45°C for 30 minutes. The secondary antibody binds the RNA-DNA hybrid-antibody-conjugated paramagnetic bead complex. Non-bound secondary antibody is washed

away using a detergent based wash buffer (40 mM Tris, pH 8.2, 100 mM NaCl, 0.1% Triton-X 100 and 0.09% sodium azide).

[00193] A substrate (dioxetane-based substrate from ABI, called DCP Star, with Emerald II enhancer) is added to the washed beads and wells that contain high-risk HPV DNA create light that is detectable by a luminometer and measured in RLUs (relative light units). An assay positive standard containing 1pg/ml of HPV DNA is used to establish the positive cutoff. All sample RLU values are divided by the RLU value for the positive standard creating a RLU/CO (RLU to cutoff value). Results are reported in RLU/CO and anything greater than or equal to 1.0 is considered positive.

Example 3: Stability Testing

[00194] Following initial testing, samples were stored at room temperature and 33°C to observe the stability of the samples. Testing was conducted as far as 21 days post collection. FIGS. 3 and 4 demonstrate that the RLU/CO value for each sample does not change with time up to 21 days. A 2x2 analysis comparing baseline results to the results after 21 days of storage and scatter plot analysis demonstrated the linearity of the RLU/CO values with time. Based on these data, it is possible to conclude that samples collected and stored at either room temperature or 33°C for as long as 21 days provide comparable RLU/CO values as tested at baseline. Using linear mixed model comparison of RLU/CO values against the temperature of storage the P values are 0.8803 for room temperature and 0.9517 for samples stored at 33°C indicating that values are equal.

Example 4:

[00195] This example describes the limit of detection (LOD), the C₉₅ concentration, and cross-reactivity experiments using re-engineered HYBRID CAPTURE chemistry and high risk (HR) and low-risk (LR) HPV plasmid DNA constructs. LOD is defined as the copy number required to identify whether virus is detected. The C₉₅ concentration is defined as the copy number required to identify whether the signal for the specimen is above a presumptive clinical cutoff 95% of the time.

[00196] Two independent assays using full length complementary RNA probes that hybridize to either HPV16 or HPV18 and HPV45 DNA were conducted. LOD and the C₉₅ concentration were determined using serial dilutions of HPV16, HPV18, and HPV45 genomic DNA and testing with the complementary RNA probes. Cross reactivity was determined using

genomic DNA from LR and HR HPV types diluted to approximately 1×10^7 copies per reaction and testing with HPV16, HPV18, and HPV45 RNA probes.

Table 5: Limit of Detection and C_{95} Concentration

	Probe	HPV 16	HPV 18/45	HPV 18/45
	Target	16	18	45
Copy No.	LOD	564	604	533
	C_{95}	8,464	8,464	7,444

5

Table 6: High Risk Type Cross Reactivity

HR Target Type	Signal to Cutoff Ratios	
	HPV 16	HPV 18/45
→ HPV 16	172.47*	0.13
→ HPV 18	0.22	93.87*
HPV 26	0.14	0.16
HPV 31	0.19	0.19
HPV 33	0.15	0.21
HPV 35	0.23	0.20
HPV 39	0.15	0.19
→ HPV 45	0.19	146.92*
HPV 51	0.15	0.24
HPV 52	0.15	0.20
HPV 56	0.15	0.20
HPV 58	0.15	0.19
HPV 59	0.15	0.20
HPV 66	0.15	0.20
HPV68	0.15	0.27
HPV73	0.20	0.15
HPV82	0.14	0.15

* where $RLU/CO > 1$ = Positive

Table 7: Low Risk Type Cross Reactivity

HR Target Type	Signal to Cutoff Ratios	
	HPV 16	HPV 18/45
HPV 1	0.32	0.34
HPV 2	0.34	0.37
HPV 3	0.39	0.39
HPV 4	0.27	0.26
HPV 5-9	0.28	0.34
HPV 5-48	0.20	0.23
HPV 8	0.27	0.33
HPV 30	0.26	0.27

HPV 34	0.17	0.20
HPV 40	0.35	0.34
HPV 42	0.15	0.15
HPV 44	0.21	0.33
HPV 53	0.28	0.35
HPV 61	0.42	0.48
HPV 62-116	0.58	0.15
HPV 62-177	0.24	0.14
HPV 67	0.23	0.29
HPV 69	0.46	0.41
HPV 70	0.14	0.15
HPV 81	0.55	0.51

Cervical specimens were collected at an external clinic site and placed into digene collection medium (DCM) per routine clinical procedure. Specimens were tested using the hybrid capture HR HPV DNA screening assay, and the reactive specimens and a subset of the non-reactive specimens were assayed using the hybrid capture HPV16 and HPV18/45 genotyping assays. A subset of the reactive specimens were also evaluated using HPV genotyping by GP5+/6+ PCR followed by Luminex detection. Any of the detergent-based collection medium described herein may be used, for example, the media may contain 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25 mM EDTA, 150 mM NaCl and 0.05% sodium azide.

Representative Clinical Specimen Data

ID	HR Screen RLU/CO	HPV 16 RLU/CO	HPV 18/45 RLU/CO	High Risk Genotype			Low Risk Genotype				
3350	366.8*	156.7*	0.28	16			Neg				
3696	283.9*	0.50	0.20	35			Neg				
3631	278.0*	328.2*	0.20	16			40				
3419	211.2*	0.12	0.15	52			Neg				
3711	205.2*	0.13	0.22	56			Neg				
3355	158.2*	0.16	0.15	51			74	83	91		
3718	154.8*	0.19	117.0*	18			Neg				
3463	141.8*	0.13	0.15	66			Neg				
3514	124.8*	106.8*	0.17	16			Neg				
3637	65.8*	0.17	0.16	68			Neg				
3576	50.6*	0.13	0.13	52			32	42	62	67	90
3656	47.3*	0.14	0.22	31			54	72			
3415	10.2*	0.13	0.15	82			28	85	86		
3366	9.6*	0.11	5.4*	18	35	45	42	86	87		
3434	8.6*	0.14	5.2*	33	45		72	87			
3229	8.4*	15.7*	0.14	16			Neg				
3705	0.25	0.27	0.10	Neg			Neg				
3239	0.25	0.13	0.12	Neg			69				

3717	0.23	0.17	0.17	Neg				Neg				
3719	0.22	0.10	0.13	Neg				32				

Table 8

Example 5:

[00197] This example demonstrates the reduction of cross-reactivity of the 17 full length (B17FL) Probe cocktail compared with the strategically-truncated probe cocktail (B17XX)

5 against eight low risk HPV types. Low risk HPV plasmid types 30, 40, 53, 61, 67, 69, 71, and 81 were used as targets at concentrations were tested: 0.5, 1, and 2 ng/mL. Results are summarized in Table 9 below.

Table 9

	B17 FL				B17 XX		
	0.5 ng/mL	1 ng/mL	2 ng/mL		0.5 ng/mL	1 ng/mL	2 ng/mL
HPV 30	0.62	0.43	1.05		0.29	0.37	0.58
HPV 40	0.31	0.29	0.45		0.24	0.31	0.45
HPV 53	0.84	1.39	2.44		0.77	1.23	1.99
HPV 61	0.40	0.49	0.81		0.33	0.39	0.72
HPV 67	0.43	0.73	1.25		0.27	0.43	0.62
HPV 69	4.01	7.90	14.97		0.73	1.08	3.09
HPV 71	0.24	0.27	0.28		0.20	0.20	0.22
HPV 81	0.37	0.48	0.90		0.21	0.26	0.49

10 **[00198]** Readout is shown in RLU/CO. All reactions having an RLU/CO greater than or equal to 1 are considered to have a high degree of cross-reactivity. As can be seen, in all cases, the full-length probe cocktail exhibited higher cross-reactivity than the corresponding strategically-truncated probe cocktail. In addition, the following full-length probes displayed a high degree of cross-reactivity: HPV 30 at 2 ng/mL; HPV 53 at 1 ng/mL, and HPV 67 at <0.5

15 ng/mL. This high cross-reactivity was either reduced or eliminated with the strategically-truncated probes.

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[00198a] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

- 5 **[00198b]** The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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The claims defining the invention are as follows:

1. A polynucleotide probe at least 1 kilobase in length, comprising at least one sequence having from 70% to 100% complementarity to at least 100 contiguous bases of each of L1, L2, E1, E2, E4, E6, and E7 of a high risk HPV nucleic acid selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 17, SEQ ID NO: 104 to SEQ ID NO: 110, SEQ ID NO: 116 and complements thereof, wherein said polynucleotide probe does not contain any sequences of at least 100 contiguous bases having 70% to 100% complementarity to at least 100 contiguous bases of a low risk HPV nucleic acid of the group consisting of SEQ ID NO: 18 to SEQ ID NO: 43 and complements thereof.
2. The polynucleotide probe of claim 1, wherein said polynucleotide probe does not comprise any sequences having about 70% to 100% identity to SEQ ID NO: 44 to SEQ ID NO: 57, SEQ ID NO: 111 to SEQ ID NO: 115, and complements thereof.
3. The polynucleotide probe of claim 1 or 2, comprising a sequence having about 70% to 100% identity to a sequence selected from the group consisting of SEQ ID NO: 97 to SEQ ID NO: 103, SEQ ID NO: 117, and complements thereof.
4. The polynucleotide probe of any one of claims 1 to 3, consisting essentially of a sequence selected from the group consisting of SEQ ID NO: 97 to SEQ ID NO: 103, SEQ ID NO: 117, and complements thereof.
5. The polynucleotide probe of any one of claims 1 to 4, consisting of a sequence selected from the group consisting of SEQ ID NO: 97 to SEQ ID NO: 103, SEQ ID NO: 117, and complements thereof.
6. A kit comprising at least one polynucleotide probe of any one of claims 1 to 5.
7. A method for generating a polynucleotide probe specific for a high risk HPV nucleic acid, said method comprising:
 - a) providing a template nucleic acid comprising a sequence at least 1 kilobase in length sharing from about 70% to 100% identity with at least a portion of the high risk HPV nucleic acid;

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- b) comparing the sequence of the template nucleic acid with at least one low risk nucleic acid; and
 - c) truncating the template nucleic acid to remove any regions of the template nucleic acid that share from about 70% to 100% identity over 100 contiguous base pairs with a region of the low risk HPV nucleic acid.
- 8. The method of claim 7, wherein the template nucleic acid is truncated by amplifying the template nucleic acid using a deletion primer.
- 9. The method of claim 8, wherein the template nucleic acid is methylated before the template nucleic acid is amplified.
- 10. The method of claim 9, wherein the methylated template nucleic acid is selectively digested.
- 11. A polynucleotide probe generated according to the method of any one of claims 7 to 10.
- 12. A nucleic acid probe set generated by fragmenting a polynucleotide probe of any one of claims 1 to 5.
- 13. A kit comprising a nucleic acid probe set of claim 12.
- 14. A method for determining the presence of a target nucleic acid in a sample, the method comprising:
 - a) hybridizing at least one polynucleotide probe according to any one of claims 1 to 5 and claim 11 to the target nucleic acid molecule to form a double-stranded nucleic acid hybrid;
 - b) capturing the double-stranded nucleic acid hybrid on a support, wherein the support comprises a first antibody specific for a double-stranded nucleic acid hybrid; and
 - c) detecting the target nucleic acid molecule.
- 15. The method of claim 14, wherein the double stranded nucleic acid hybrid is a DNA:RNA hybrid.

16. The method of claim 14 or 15 wherein the target nucleic acid is a high risk HPV nucleic acid.

17. The method of any one of claims 14 to 16 wherein the sample is a biological sample.

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FIG. 1**HPV33 Deletion Probe Template (SEQ ID NO: 104)**

CTGTAAAAAAATATGGAAAACGTTTACGCCTGCGACGTAAAAATAAAATAAC
TAGGATGTAAAAACAAAGTCAGCACCGTCTACAACAATGGTGTCAAAAGGAA
AAAAAGGCGAAATAGGAACAAATGGGCTAGATGTGGGAAATAAAGGGGAAG
GTATATCAGGGCCAGACATAACAGGAGTATCAAATCCTGTATTTAAAGGTAT
AGACACATTGCTGGTACGTGTTGTTGCAAACGTA CTGTATGAGTGTTCATTG
GGGTGTGTACATTATCCACATCGTCAGCATAAACATCATACAAACCATCATT
ATACTATAAGACGATGTAGAAGTATCATGTAAAGGCTGTAATTCATATTGTTT
ATTTGGCACGGTGTGGTCTAAAGGCACAATAGGACTTAAATCCTGATAATAA
TGTATTCTAGCTCCAATTTGTTTACCCTGCGAGTTTTAAGTGTGGCTTTTTGA
CCTACTCTACTAAAACGCACAGTATGTCTACGAGATGTAATAGCAGGCCTAT
GTAATGCAATAATATCTAGAAAGTCAGGATCAGGAGCAGGTGATATATCACT
ATGTTGAAATTGTAATGTGTCTTCAGGGTCAAAGCTTTCAAATGCAGGATTAT
CATATGTTATAAGTTTATGAGGCGATGTTAAAAAAGCAGGGTCAACAACCTT
AACCTGTTGGGTATTGCGACTATATAAACCAAGGCGTGCCACAGGGCGAGAC
CCTGGAATGGGCGTGCTTGATGTTACATTACTACTGTCTGTGGAAACAACAA
AGGTATCCATTGGTATGTTTTTCATAACTTTGTGTGCTAACAGTAGGGGAAGAA
AATATAAAATGTCCAGAGGCTTCTGCAGGCGCTGGAGGGTGTAGTACAGATG
GTTTCAGTAAATGTGGGATTTAAATGTGTAGAAATAGTTTGAATAGATGACTC
CCCAACAGATGAAACATTAATAATTGCAGGTGTAGTATCTGCAGATGTAGTA
ACATCAAAACCTGATGGTGTAGGAATAGATGGGGCTGGTGCACCTGCCTCTA
TAAACTTGTCTTCTTCTATTAATGACACTATAGACGAGTCTAAAGGTCCAACA
GTGTCTACAGTAACCGGAGGACGTATAGGCTGCAAGGGGATTGCAGCTGTAG
GTGGGTACAGTACCAATAGGTACATAGCCAGTCCTTCCACCTGAACCAGAGCC
TGTGCCAATACCTAAACCACCAAAAAAACCCTAAACTGCCATATTTAAGA
ATTTGATCTGCTATGGTACTTCCCTTCCACTTTAGGAATAACATCGGGTGGGCA
GGTGCCTGTGGCCTTGCATGTTTGGTATAGTTGTGTTGCAGATGCACGCTTGC
GCCTTGTAGATCGTTTGTGTCTCATAATAATACACTGCTAAAATATAAAGGTA
TTTATTAGTAATACAAAAAATAAATAAATAAACAACAATGTAAA
ACACCACCATGTGCACATATATACAAACAATATATACATGTATATACATTACT
CTTGTTGTGTCATATGCTGTGCATGAAAATTAATACACATCATTGGTAAATAT
AAAAACAACAATAGCAAAAAAATTTTTAAAGGAGATCCCACAAACACC
CAAAGCAGCAATACCAACACCTGCAACCAAGCATAGGTAGAAATGGAAAGT
ATTAAAGGACGTAATAATAAGGATAAGCATAAAAAACAATATAAAACATAAT
ACAAAAACAATATCATGGTTATATACAATTAGCAGCACATATTGGCTTGTG
ATGTACACTTATAATGTCATAAATCCAGTACTTATTTGCACAGTAGGTGGTAT
TTTTACGGTACCTAAAAACATTTGTTGTTGCTGTTTCAGTTACAAATGTTACAG
TTACAATTCCATTTTACTATTTTGTGTTGCTGTTTCAGTTACAAATGTTACAG
ACATAGA ACTATACAACTCTTTATAAGGTTTTAATCTGTATCTTAAACATTTT
AAACTATTTGATTCACCTTTTAAATGCACTATAGGTGCAACGTTAGGACTACA
CACAGTCCGCTGCTTGTGTTGTGCAGTTAGTTGCAGTACGTGCTGTTCTATTGTC
CAAGGCGGGGTCTGCACAGAACAGCTTTGTAAGGGGCTGGGCGGTGTCTGTG
GTGTCTGCAGGTCGTCGTCGTTTGGCCGCTGCTTGTGGTGGTTCGGTTATCGTT

GTCTGTCTGTATGTCAGCAGTTTCAGTAGTGGATATTTGGTTGCTAGATATAG
ACGTAGGACAAACAATTACCTGACCACCCACATGTACTTCCCACATTTGTGTT
TTAGAATACTTTGCAGCATCCTCTTTAAAATATTTAAAATATACCTTTTCACA
GTTATGTATATAATACATACCTATATAATCTACTTTCCCTGTAACCATAGTAC
ATGTATCTTCTCTATAATATATATTTACCCCAAGTTTGTATAATCCATTGTAT
TTTTTTTGTCAATTGTCATATTGCACAGTTACTGTTTCTCCTTGTTTTTAAAACA
TTTTGGTGGTTCACAAAGCCACACCTCTAAGCTTGTTTGTTGCAATGTCCATT
GGCTTGTA**CTATACTGTGATTACTTAAATG**tctctaagccattgtagtcaattactgaaatgct
ttggctttgatgctaacaagaaggcaccacctgggtggcataaatgtgaaaatccattgtttggctgtatacaataaagcacact
ccatgcgtatcagttccaatgttcaattgtgatggtaaatcagtttatcagcttcgtaaagagctagcattttctctgcaactgcaatt
aaacgtgctgatatttctccatggtttcctgtctctctctattaaatctaattgcaccacgtccttgagaaaaaggatttccaat
ttcatcattattgcatacactgggttaccatttcatcaaatgggaatggatttttaattcaataactgttaactactatgtaaatag
gccatctagagtctgtgcctgcattgtatttgaggtaagaagcagtggtggacattttaattgcactaatgccctatgtttcacatcta
ttgaaatttcatttccatctaacgcatttctcatgtaactatctatatatgtccaactattggcggtacatcatctatctatctattttgca
tctgataatggctgcaaccaaagtgcattttagaatttacatgatataacacacccctttaaaaactgtattaaactcattccaaaa
tatgactttctgtattgtgtccacaaattagcatacagcttttttggtataccttttaaaacttttaaatgcacataaaatgct
gtaaattcaatgtttgatcttaacaactgtactattggtctcaatttctccatcattgtttttcacatctactttgtatccattgtct
attgacattttacgtttttctgtttttataatgtctacacatttccacagtcctttactattttgcttgtagtacttttaaaatgcag
cagcattactattgaatctgcaagttgtgcataataatgcaatgtcactatcgtccgttaactcgttatcatatgccactgtaccat
ttcacttaatacaatatattatcattaaagctatgttgtaaaacaggttagtctatctatccattcaggtgttgacctgtacatcactaat
gtttgacattgctgttctaaccaatacaatgcacatgtttggctccgtaattttgggtggctctataaccatacatgtttcaggtattgat
aataaattactcattagttttgctactgttaacctgtttgtctacacctaatacttaatacaataatattatttctctatcgcgaagtta
aacattgtaaatgagatacaaaactatgctgttaattaatacttttaacttttctgactgatggactaattccatactcgtttatacac
caatctgtacagctgttttatcacttttaaatg**TCTTACTAATTCATAAACTTATTCCATAGG**
CCTCTTTAAATTTATATAATATATTTGCTTTTGTATTACTACTATGTAGAACAT
TACTAATTTCTGCAACGTAACATTTTCACAGCTATCTACATTTGTCTCACAG
CTTACTTCTGAATCATCCCCACCCCACTAGATTCTAAGTCATTTAAGTTTGTG
TCGCCATTTTGACTTTCTACCTGTTGTACCATCTGCTGAGTTTCCACTTCAGTA
TTGCCATATCCGCTGTCTTCTAGCTCATCTATTTTTCGTTTCTGTATGTGCATT
CTTTATTTTATTAATAGACGTTCTACACGGGTTTGCAGCACGATCAACAACG
TCCTCCGCAGCACTTTGTGAACATGCGGCAAACTTTCGTTTGTAGTGCACACAC
AGCATTTAAATCATCCTCCCCCTCCTGTATATTAAACAATGCCCGGGCTGCCT
CTGTGTCTGCCTGTATACTATTTTCCATAGAATCATCTATAAACTCTAGTAAA
TCCGTGCCACTGTCATCTGCTGTTTCATCCTCATCTTCTGAAATATTATCTCCT
GTTCTTCTCTATGACTGCTTCTACCTCAAACCAACCAGTACACCCCATCCC
AGCCCCATTTGTACCTTCAGGATCGGCCATTGTAGATGATGTTTATTGTTGTG
CACAGGTAGGGCACACAATATTCAGTGTGCCATAAGTAGTTGCTGTATGGTT
CGTAGGTCACCTTGCTGTACTGTTGACACATAAACGAACGTGTTGTTACAAGT
GTGACAACAGGTTACAATGTAGTAATCAGCTGTGGCTGGTTGTGCTTGTCCAT
CTGGCCGGTCCAAGCCTTCATCCTCATCTGAGCTGTCACTTAATTGCTCATAG
CAGTATAGGTCAGTTGGTTCAGGATATAAATCTAAAACATATTCCTTTAACGT
TGGCTTGTGTCCTCTCATGGCGTTTTTACACGTCACAGTGCAGTTTCTCTACGT
CGGGACCTCCAACACGCCGCACAGCGCCCTGCCCAACGACCCGAAATATTAT
GAAATCGTTTGTTTAAATCCACATGTCGTTTTTTTTTCTTGAGGACACAAAGGT
CTTTGACATATAATACACCTAATTAATATTTTCAATTAAGGTTTTTTAACTGTT
TGTTCTAATGTATTTCCATATACAGAATAATTATAATGTCTATATTCATAATT

TTAGATAAGAACCGCAAACACAGTTTACATATTCCAAATGGATTTCCCTCTCT
ATATACAACTGTTAAATCTGCAAATGCAAAATCATATACCTCAGATCGTTGCA
AAGGTTTTTTGCATTCCACGCACTGTAGTTCAATGTTGTGTATAGTTGTCTCCA
ATGCTTGGCACAAATCATGCAATGTTCTGTGGTTTTTCCTCAGTGTCTTGAAAC
ATAGTCGTGCAGTACCTTACTGCAAAATGGTTGCTTTATATATGCACCGTTTT
CGGTTGAACCGCTTTTCGGTTACACCCTACTTTTTTAAAACCTGGCATTATAGTT
TACTATTATATAAAAAGATTATAAATGACCAATATGACCTAAAACGGTTAGTC
CACACCTGGTAATAAACAGGTAGTGACTCACTTTTACAAGGCATAGTTTGGC
ATACAAAGCAATTGTGTAAGCCAAAACCTGCACTTAAGGTTTGAAAGTAAAGT
AAAAATATGTGCCTAAAAGCATGTGTAAACTATTAGTACAATTAAGTATTTTG
CAATTGCACTGCATGTATATATGAGTCAATGCAGCAAGTACAGACAAACACA
GTGCAGGAAAGAAAAGGATTAAGTGTCTGCCAATGTCATAGGGTATGTATG
CCAATGCAGCAAGTACAGACAAACACAGTGCAGGAAAGAAAAGGATTAAGT
GTTCTGCCAATGTCATAGGGTATGTATGCCAAGTAACCGAAAACGGTTAGGC
ATACAAAATGGAGGAAATTGTACAATATGGACACTAGTATGTCAAAAGGTAA
AGCAATACTAAACATGTAGGTACTACAAATATAGGGAAATAAAGGTAGGTAC
ATTGCAATGCAGGGTAGGGCAAGCAATACAACTCCTTACTCATATAGGTACA
CCCATATACATAACATACATACAAATAGTTTAAACAAATACACACAATGTTTTA
TTAACATATACACAAACAGGAAACTGACAAGTACATAGAACATGCACACAA
ACAAGTACACATAAAAACAAACACAGTAACATACAAACATAACATACATTGTAA
CACATACACAAAAAACAAACAACACAACACAACACAACACAAGTACATA
GACAGAACAAAACAACATAACACAATTACACAAAGTGTTATTTTTTAAC
CTTTTTGCGTTTTGCGAGCGATGTGCGGGTGGATGTGGGGGCTGCACGTTTAA
GTTTAGGTTTTGCTTTAAGACCTGCCTGTAATAAAAACCTTGCCTCCCAAAGGA
AACTGATCTAAATCTGCTGAAAATTTTTCTTTTAAATCCACTTCCCAAATGT
ATATTTACCTAAGGGGTCTTCCTTTTCTTTGGAGGTACTGTTTTTTGACACGT
AATAGCCTGAGAGGTAACAAACCTATAGGTATCCTGTAAACTAGCAGATGGA
GGAGGTGTTAAACCAAATTGCCAATCTTCTAAATATCTGGATTCATAGCATG
AATATATGTCATAACTTCTGCAGTTAAGGTAACCTTGCATAGTTGAAAAACAA
ACTGTAGATCATATTCTTCAACATGTCTTATATATTCTTTAAAATTTTCATTTT
TATATGTACTGTCACTAGTTACTTGTGTGCATAAAGTCATATTAGTACTGCGA
GTGGTATCTACCACAGTAACAAATACCTGATTGCCCCAACAAATACCATTATT
ATGACCTTGTGCACGTTGTAGCCAATATGGCTTATTAATAAAGTACTGAGATTCCG
AAGTAACCATTGATCCACTAGGAGTGGGAAAAAAAGCACTGCTTTGAATAGA
GGCAGTAGTTCCTGAACCTTTAATGTACAGGTCATCGGGAACAGCCTCTCCTA
ATGTACCAGCCCTATTAAAAAAGTGTCTTACAAACATTTGTTACGTCGAAGA
AAGAAAAATAAACTATCACCATAAGGCTCACTAGTCATTTTTTAAATAATCTG
GATATTTGCATGTACTGCCACAAATATCAATAGGAACATCACTTTTATTAGCC
TGCGATGTTTTTAAAATCCATGCAACCAAATCCTGTGTCCACCATATCACCATC
CTCAATAATAGTATTTATAAGTTCTAAAGGTGGACAATCATTGGCAGGTGCTG
CATTAGTACAAGCAACACCTTTACCCCAATGTTCCCCTGTTGGAGGCTTACAT
CCAAGTAAACATAACTGTGTTTGTATAATCCATGGATAAACATTCCCTATT
ATCAGCACCCGGTTGTCCAGGATACTTGTTACCGGTTTCAGTGTCAATTAATT
TGTTTAATAAAGGATGACCACTTATGCCAACGCCTAATGGCTGCCCTCTACCT
ATTTCAAGGCCTACACATGCCCATACTAATCGTTGTGTATCAGGGTTATAAAA
GGAGGTGTCAGGAAATCCAAATTTATTAGGATCTGGTAAACGGACCCTAAAA

ACCCTATATTGCAAGCCTGATACTTTGGGTACCAATAATTTTTTAGCGTTAGT
AGGATTTTTAATAGAAAAATATGGATGGCCAACAGCAAGAAGTCTGGAACTA
CCAGCATAATAATAAATGCTTGTGCGAGACACATATTCATCAGTGCTGACAA
CTTTAGATACAGGTACAGGAGGCAGGTACACTGTGGCCTCACTAGACCGCCA
CACGGACATC

FIG. 2**HPV39 Deletion Probe Template (SEQ ID NO: 105)**

CTGAAAAAAAAATAGGGAATACGTTTCCGTTTTTTTAGGAAAAAAAAATACAATAA
TGGCAACAAATAATAATTGGAACCCTGAATGGTTATTGCATATGTTGTGTCTA
TTGGTCCAGAAGGCACCAATGGCAACTGTGGAGTAGTACTTGGTAAAGCAAT
ATCAGGACCAGTATTTACAGGCATATTCCATGAGGTACTAAAAGGAATAGTT
GTATTGGCATATTTAGTAGATGCTGAAGAAGCCACAGAAGGTAGTGAGCCTG
TGTTATATGTAGTGCCCGAATCCCTTGTATTATTAAATGCAGTATCTAAATAT
GTGTTATTGTCCACATCAGCATATATATCAAATAATGCATCTGAAGCATCAGA
GGGCTCAGCGTGAAGTGGGGCTGTAATTCAATGCTTTCAGCAGGAGCAATA
CTACTAATGTCATGGTAATAATGTACTTGCCTCCAATTTGTGTGCCACGCCG
GGTAACCATGGTAGCCTTTTTGCCAAGCCTACTAAACCTTACTGTTTCCTTTAC
GCGAGGTTAAGGCAGGCCTATGTAAACGAACAATGTCCAGAAAATCCGGATC
TGGAGCTATGTCAGCAGCTTCATATGTTAATGTAGTATCAACAGGCTCAAAA
GCAGGATTATCAAATGTTACAAATGATGAAGGGTGAGTTACAAAATCAAAT
TACTAACACGAACCTGCTGATGTGCTCTACTATATAAACGTGGTCCTGCCACA
CGACTGATTCCAGGTGTAGGTGTGCTGCTAATAGGTTCCGTACCTGTGCCATG
TGTGGCAAACACTTCCATAGGTATTTCCCTCATAGCCATGTGTACCTGATGTAG
GGGTACTGACAAATATATTACCCGAGGTTTCACCTGTTTGGGGAACCTCAATT
AAGGAAGGATCCGTAAAGGCAGGGTTAGTATAACTAGTAGAGGTTATTTGTA
CAGACCCAGAGGAGGGTGTAATATTCAATACCGCAGGCGTAGTAGTAGAAG
AAGAAGTAATTTCAAATCCAGAGGTGCCTGTAAATGTTGGTACTGGTGTTCC
AGAGGTTATAACACTTGAGTCCTCCACCAATTGCACAATAGATGGCTCAGAA
GGACCAACAGGTTCAATAGCTACAGGTGGACGTGCAGGAGACACATCTACAA
CAGTATTAGGCCTACCCCCAGGGGTATATATCCTGTGCGTCCCCCAGTACCA
GTACCTGTGCCTATGCCTAACCCACCCAAAAATATACCTAAACCAGTCCACT
GTAAAATTTTGTGTCAGCAAGTGTAGTACCCTCAACTTTATCAACAACGTCTGGT
GGACAGGTACCCGATTGTTTACAGGTTCTATATAGGTCAGTTGCAGATGCAC
GCTTACGCTGGCAGCACGGTGGGAAACCATGTTTATTAGTAACAAAAAAA
ATATAAAAAGCTATGTACAGTAGTTATGCACAATGCACATACATATACAGTA
CTATATCATATCCATTGCCAGTCTATGCAACAACCACATGGGCAATACAAAA
AATAGTAAATATACAAAAAACACCTCCAATGGTGTGGTACGTATAAGAATAA
ACACAAACACAATTATCCACACATACGCACACACATGCACAGACGGCAAAA
GCGGGACATTGCAACATATATATATACACACACCAAAACCACACCAAAAATAC
CAATAATATCATCTATGTAGTATGTATACAATATACATACACAATACCCATAG
TATACTTTACAATGTCATGTAACCCAATGAAACATGTACACTAGAAGGTATTT
TAACAGTGTCCAGAAATTTTTGGCGTTGTGACTCTGTGGCATATGTAACAGTT
AATATGCCAGCGTTTTTGGTTCCCTTACCCCGTATCCAATGCCAGGTACATGA
AATATTTTCAAACAATGTGTCATATTTTTGTAGTCTATATCTTAAACATTTTAA
ACCATTTTTGTACCTTTTAAATGTATTATAGGCGTAGTGTTACCACAACCTGA
GGTACCGTCTTGTGTTGTGGCCTGTACTGTTACTGTGGAGTGGGTTGTTAAGA
TGGTCCAGGGACACTCCGTCGGGCTCAGTGGGCTCTGTGACTGCACACTGTC
GAGGTCGCTTTCGAGACGGCGGGCGGGATTGTTTTTTGGGTGCATGGGGTTGTT
GCGGTGGAATGGGTCGCGGTGGTGTGTTGATAATTCGGTAGTAAGTTcagtagt
gggtaccgatccgtcactggtactgcacatagagtccggacaatgaattatgttgccattataatgcacttccatttgcactagtc

ccatacctttccgcatcttgaataaacacttcatagtatacttttaggtgctcgttcatataatataacccaatagtcacacaccctt
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cctccactgtagttcctgttttttaaacattgtttggctgtgtatgccacagttcattactagtgtcttttaatgtccactcctctgtatt
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tattgattactgtcttgcatagtattctagtatttgcctgtaacacatttaaacgttgtgaaagtgtttcatcattgtctcctcatcct
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tcaaatggaaatgcattaggaaatttaaacactgttagcctactacgtaaatatggccacctatcgtctccacaggattggtattgg
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cgcttgaatgtttacacattgttgcacaatctttacatatatttgcctggcagttacttttaaaaaggctgcagcattactgttacaatc
gtaacattgcataaataatgctatgtcactttcatcagtatattcattgtcaaatgccattgtaccatgtccgataggtaaataca
ctatcatctattccattgtgtataacagttaatcgttgcattctggtcgtatccctgttaccacactaatattggatatacctgtgctg
ataccaatagtagtgcctacagggctgcgcagtttaggaggtccagaagcatacaacttttggacatgtaacaatgtactta
atcccttctacagtaaccctatttttccacatgtatatctt**ATTAGCATTAAATTAGTACTCCTTGT**
TTTGTGTCTAAGCTTTGTATATGTGTATATAAGGCATATTTGTTGATTAATGTT
TTAAATCCTTCTGCAATAGTTGGATGTACTCCAAATATGGCTGCCACCCAGTC
TGTACATGTTGTTTTATCACTTTTAAACGTACGTACCAGGTCAGTAAAGGA
GTCCATATGTTTCTTTAAATTGTGTTAGCATTGCAGCCTTTTTGTTATTGGATT
GTAACAATAATTTAATTTGTGTCAGTTGGAGATTTGGGATCCTGGTTTTCACTA
TCTATAGCACTATCCACACTACTACACTCCTCCCGTACACTGCCGCCATGTT
CCCTTCAGCATCCCCATTTGTATTAGTTGCTACAGTTACCTCCTCCACTTCAGC
TGTTTCCACTTCCATATTGCCATATCCGCTGTCTGGCACGGAATACACCGTTT
GTGTTGCCTGCGTACTGCTTACATTTAATGAAATTTCTGTAGTGTTCCTG
TATTCCTGCCTACTTTTTTTCCATACGGTCTAGTGTGCGCACTGCTGTCTGTAT
ACTTTCGTTTTTAAGGCACGCACTGCTTGTGCATCCCTTTGGGCCTCTTG
CATATGTAAAAGTACCTGTGCTGTCTCACGCTCTGCCTGTACACAAATATCTGTGGAA
TCATCAATAAAGTCTGCCAGGTCTGAACCTGTATCTGTTGCATTTTTCATCCTC
ATCCTCCGACACTGTGTGCGCTGTTTGTATCTACTATTGCCTGTACTAGAAA
CCATCCGTTACATCCCGACCCATCCCCGTCTGTACCTTCACGATTGGCCATAG
CAGGTTACTGGTTTGCAGTTGCACACCATGGACACACAAATCCTAGTGAGTC
CATAAACAGCTGCTGTAGTTGTGCGAGAGTATCCCGTGAGGCTTCTACTACCA
GCTGCAGTGTGTTGTTACACTTACAACACGAACACTGCATTGTGTGACGCTGT
GGTTCATCCCGTCTGGCTAGTAGTTGATGTTGGTGATTAACTGCATGGTTCGGG
TTCATCTATTTTCATCCTCTGACTCTCCTAATTGCTCGTGACATACAAGGTCAAC
CGGCTGTATTTTCATTGTAAGGACATAAATCTAATACAATTTCTGCAAGGTGG
GCTTTGGTCCACGCATATCTGATGTTATACTTGGGTTTCTCTTCGTGTTAGTCT
GCGGTCCCTCCCGTTTTGTGGTCCAGCACCGTCGACACTGTCCTGTATAGCTTC
CTGCTATTTTATGAAATCTTCGTTTGTCTATTTAGGTGTCTTAATTTTTCTGCTG
GACACAGCGGTTTCAGACAACACATGCACCTTATTAATAAATTATATAACTTT
GTATTAGTTATATTTTCTAATGTAGTTGCATACACCGAGTCCGAGTAATATCG
TAGTCCCGTATTTTAGCATAAAATTTTATACATGATTGGCATGCAGCTAGTG
GTTCCCCGTCCCTATATACTACATATAAATCACTAAATGCAAATTCATATACC

TCGGTTTGCTGTAGTGGTCGTCTGCAATAGACACAGGCTATTGTAATGTCCTG
CAAGGTGGTGTCCAGCGTTGTGCACAGGTCTGGCAATTTGTATGGCCGTTCTG
CAGGATTGTGAAATCGCGCCATCGGTATGGACAGAACTGTGACTGCGTTTT
ATATCCACCGATTTTCGGTCCTGACCGTTTTTCGGTTACTCCCTTTTTTTAAACAA
GATACTTATAAAATGTTATAAGTAAAAGTATAGGTATGTATGCCAACCTATT
TCGGTTGCATAAACTATTGTGTGCTACATTAGTCACTTGTATAACTTATACTG
TAAGACACATTCCTGAATCAAAAAGTAGTTATACAGGCCAACTATTTAATTA
AGCAAGTAAAAACAGCTAAAATATATGCCTAAAAGCAGTTTTATTACATAGG
GTGGAGATATGGATGTTGCCAAAGTATTGTTGCAGTGCACCTGGACAGGATG
ATGAGTAATAAGGCGCGCCAGTAAAAGTGTACCAGTGCCAACTATGCAGGG
TGAACCTAAGGTAAAGACATGTTTTTGCTGAGAAAGGTGGTTTTCCACTAGTTT
AAAAAAAATGCTCAACCCACGACCGACTTCGGTCGCCACAAAATGGCGAAGT
ATAAAATGTAGAAACGCGTACAACAATGTAAAAATTACATATTAGGTAGTAA
ACACACCTTAGGGTAGGGCATATAGATTATGTATGAAATGTTGCAGACCTAT
TATATGTAAAACAAATACACTGTTAGTCAACAGGGTGTGCAATCACACATGAA
ACTGTCATACATACTTTATTAACAAAAACATATACACACATACCTACAAACAT
ACACATATACACTCAACACATAAGGAAACAAACAACATACACACACATAAC
AAGGCATACACATGCATTATTTAGACACACGTTTACGTTTGTGTTTAGTAGCT
GAGGACGAGGAAGTGGATGCAGCAGGCCGCTTTCGGGGACCTATGGTAGGG
CGCCTGCGGACCCTGGCCTGCAACAAAAATTTACGTCCCAAAGGGAATTGAT
CAAGTTCCAAACTAACTTTTCCCTTAAGTCAACATTCCAAAACCTTTAGACCG
TCATATGGATCTTTCTTTTCAGGTGCTGGAGCATCCTTTTGACATGTAATGGCT
GCAGACTGTAGGTATCTGTAAGTGTCTACCAAAGTGGCAGATGGTGGAGGAG
CTACAGCAAAATTCCAATTGTCCAATATAGAGGAATTCATAGTGTGAATATA
AGACATAACATCAGTTGTTAATGTGACAGTACACAGTTGAAATATAAATTGT
AAATCATACTCCTCCACGTGCCTGGTATATTCCTTAACTTAGAAGGATCATA
TGTAAGAGGTATGGAAGACTCTATAGAGGTAGATAATGTAAAGTTGGTACTA
CGGGTAGTGTCCACAACAGTAAGAAATAATTGATTATGCCAACATATACCAT
TGTTGTGGCCCTGGGCCTTATGTAGCCAATAAGGCTTATTAAATAACTGGGAA
TCAGAGGTTACCATGGAACCGCTGGGAGAGGGGCAGTATACAGAACTACCG
GGGTTTGCACGTATATCTGTGCCCTTAATATACAATTGGGCAGGAATGGCG
TCACCCACCataaccaccagattccaaaatgtcttgcaaacagttgttcctacgtaaacagaagaacatactgtcccc
atacacatctgcagacatttgcaataatcaggatatttacaaatggattgacaaatatctaaaggcacctcacttttggttctctgca
atgcaccaaagtccatagctccatagccagTATCAATCATATCACCATCCTCAATAGGGGTG
TTTACTAGTTCCAAAGGAGGACAGTCCCCCGTAGATACATTATTGGGCTTGCA
TGCCTTTCCTTACCCACGTGCTCCCCAATGGCGGGAACACAGCCTATAATGC
ACAAGTGTGTCTGTTTATAATCCACAGACACATTATCCCTACTGTCTTATTG
GTGGTTGATGAAAATGGTGAGTTTTAGTATCATCCTGTCTATTATATAATGG
GTGTCCACTAATACCAACACCCAATGGCTGGCCCCCTGCCACCTCCACCCCTA
CACAAGCCCATACTAAACGTTGTGTTTCTGGATTATATAAGGATGCATCTGGA
ATACTGAATTTATTAGGATCGGGCAATGTCACGCGAAATACCCTATATTGATA
TGCAGACACCTTTGGAATGTCCTGCTTGCGACCACCATTCATACCCACTTTAA
AATATGGATGTCCTACTGTTAATAATCTAGAGCTGCCAGCATAATAATATATG
CCTGTGCGTGTAACATAATCATCAGTATTGACAACCTTCGCCACAGAAGGTG
GAGGCAAATACACCATGCTGTCACTAGACCGCCACATAGCCATC

FIG. 3**HPV52 Deletion Probe Template (SEQ ID NO: 106)**

CTGTAAAAAATATGGAAAACGTTTACGCCTGCGACGTAGTAAAAAATAACT
AGGATGTAAATAAAAATCTGTACCATCAACAATAATAGATGTAGATGGAGCT
GTAGGGGCTATAGGAACAAAAGGAGTATGTGTGGGTAACGAAGGTAATGGA
ATGTCAGGACCTGACTCAATGGACATAGTGGGTTGATATACAAAGTCAATAC
CACTATTAATAGGTACAGTGAAAGTATTATTATGGGTAGAAAGTGTGGAAGG
TAAGTGAAACGTGGGTTGCTGCAAAGAATCTGCATACACATCATAACCA
TCATTAATAGTGTAAGGGGACACAGACTGTGGTAATAAAGGTTGCAATTCTA
TGTCTTCCTGAACTTCAGCAGGCTGGATAGGACTAATATCATGATAATAATGT
ACCCGTGCCCCAATTTGTTTTCCACTACGTGTACGTAGGGTGGCCTTATTACC
AAGCCTGCTAAACCTAACAGTACCTCTTCGAGAGGTTAATGCAGGCCTATGC
AAAGCTATAATGTCTAAAAAATCAGGATCCGGTGCAGGTAAAAGTTGTGAAC
GATCAAAAATTATAGTTTCATCTGTATCAACGCCCTCAAAAACAGGATTGTTA
TATGTTACTAATTTCTGTGGTGATGACATAAAAGCAGGGTCGACTACCTAAC
CTGTTGTGTGGCACGGCTATATAAACCAAGGCGTGTCTAGGGCGAGACCCT
GGAATAGGTGTACTACTTGTACACTGCTGCTGTCAGTAGAGGTAACAAATG
TATCCATAGGGATTTCTTCATAGGTGTGTGTACTAATAGTTGGACTAGAAAAC
AATACATGACCAGATGCTTCTGCAGGTGCCGGGGGCTGTATTATAGATGGTT
CAGTGAATGTAGGATTTAAATGTGTAGAAACTGATTGTACAGATGATTCACC
TATAGATGTTACATTAATTATTGCAGGAGTATTATTTGCAGATGTTGTAACAT
CAAACCTGTTGCTGATGGAATAGATGGAGCAGGTGCGCCAGACTCAATAAA
TGTTGTTTCTTCTATCATAGAACTATAGATGGTTCTAAGGGACCAATGGGTT
CTACAGTTACAGGGGGACGAATGGTGGACGTGGTAATACTACTAGTGGGAGG
ACGAGTGGACAATGGCACATAGCCTGCCCTACCACCAGAGCCTGCACCTGTA
CCTATACCCAAACCTCCAAAAAACACCCCTAGGCTGCCATATTTTAAAAGTT
GATCTGCAATAGTTGTGCCTTCCACTTTAGGAATAACATCGGGGGGGCAGGT
GCCAGAGGCTTTGCATGTTTGATATAGCTGTGTTGCAGAAGCACGTTTGTGCC
GTGTAGACCGTCTGTATCTCATTGCGACAAGCTATTAAAAAATATAAAAGTA
TTTATTAGACAACTACAAAAGTGAAAAAGTATATTTTGCATATATGCATGTA
GCCAATCTACATGTACAGTTATTGCAGTTGTGCCAAATACTGTGCATGACAGT
GAATACAAAACATTGGAAAATATAAAAACAGTAGGTACAAAAAAAACACTT
TAAATGGTGACCCAATAGATACCCATAGCAAAAAGCACCAGCACCAACACCTG
CGCATACACCGATATAGATAGCAAGAGCGGCCTAAGCACTGCACAAAACAC
CATAAGCAAAATAAAACAAAATACAAATAATCCTAACATTGTTATATATACA
CAATAGCAGCAATATTGGCTTGTGTTGGGTGTTTACCATACACATATACATAT
ATACATATGTACAAATATCACAATGACATGACACCTTGTATAACTTGCACAGT
ATTTGGTATTTTAAACAGTTTTTAAAAATTGTTGACGTTGTGTTTCATCACTGTA
CGTTATTGTTACAATACCTAGTTTATTATTTGTACATTCATTACTGGTCCAATG
CCAGGTAGATGAAATTTGAACATACAACTTTTATGTGTTTTTACCCTATATC
TTAAACATTTTAACTATTAGGATCACCTTTTAGGTGTATTATAGGTGCAGTA
CAAGTTGTATGTGCAACCCGTCCTTTGTTTGTGCACTCAGTTGCAGTGACGAG
TCCCCGTGTAGTACTGTCCACGGATTGTTGTCCCCGCAAAAGGTTGTTGGGGT

ACTTGGTGTCTTCTGGAGTCTGTGACGTCTGGTCGTCGTCGTTTCTGTGGTGGTT
GTAGGTGTGTGTCTTTGGCACCCACGGACACTGCGGAGGTCTTGGAGGTTTCG
GTGCATAGGTGGACAGCAGTTTCAGTAGTGGATACTTCGTTACTAGATACAG
ATGCAGGACAAACAATTACCTGACCACCCACATGTACTTCCCATACTCCTGTT
ACACAATATTGCTTTGCATCGTTACTAAATTTTACAAAATATATTTTTTCTCCA
TCACACCAATAATATAACCCATAGTAATCTACTTGTCTTCTACAATTGTACA
TTCACACTCACCAAGTAAATAAATTTTCTTccagtttgataatccatagtattgtttatcattatcgt
attgcactgttattgtatacccatgtttttaagtaattttgtggtctgcacgccacatttctagactgtttgttgaatgtccatccatct
gtgctatattgtgtttgttaaatgctccaatgccaatgttagttcaatagcttggcaggccttgccttagacactgccattggtggc
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gtgcgttaggtcattactatcagcttcgtatagatctagtatttttctgcactgcatttaacgtgccggtatcgactccatcgtttc
ctgtcctcttctgtattaaatctaatttgcaccacgtccttgagaaaaaggattccaatttctgttgaatttcatatagaggattgcc
attttcatcaaatggaaatgggttttgaaatgaaacacaaccaatctactatgtaaatatggccacctaggatctgttctgcatttgt
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caaagctatgctgtaatactgtttgttctatccattctgggtgtaccatataccctactaatattagacaaacctgttctatacca
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AGCTGTGACATTAGTTTGGACACTGTTAATCTGTTTTTCCACATTTAAACCT
AATTAGCAGCAGTATAAGCACGCCTCTGTCACATGTAAACATTGCAAATGG
GCATATATGCTATAGGGCTGTATTAATACTTTTAATCCTTCTGCAACTGATGG
TGTTACTCCCATTCCCTATAATACACCAATCTGTACAACACTACTTCTATTACTTTT
AAATGGTCTTACTAATTCCATAAAGCTAACACCATATGTTTCTTTAAATTTAA
ATAATACAGTTGTTTTTATGCTATTTTTCGCACATAATATTTTGTATGCTTTTTA
GCGTTCTATTACTATTTTCTCATTGTCCTCTATACTAGTACAACCTTACATCTG
AATTACTAGCCCCACCCCACTTGATTGACTACTGTACTTTGCCAGTCGCCA
TTTTGCCCCGTCTACCTGGTCTGCCATCTGCTGCGCTTCCACTTCACTATTGCCA
TAGCCGCTGTCTTCTACGTGACATGGTTTGGTAAAACACACTCTGT
ATTTACACAAATGTGTTTTGCACGCGGACTACCATGTTTTTCTACACCATCTT
GCCCAGCACTTTCCGGACTGCTTGTAACCTTTCGTTTTACTGCAGACACAGCA
TGTAATCATCCTCCCCTTCCTGTGCATTAAACAATGCCCGGGCTGCCTCATG
TTCTGCCTGTTCAATTATTTATATTTGAATCATCTATAAAATCTATTAGATCTGT
TCCACTATCATATGCATTTTCATCCTCGTCCTCTGAAATGTTATCTCCTGTTTG
TTTTTCTATTATTGCCTCTACTTCAAACCAGCCTGTACATCCCTCCCTTTCCGCC
CTCTGTACCTTCAGGGTCCTCCATTGCAGGGTGTGTTTATAGCCGTGCACAGCC
GGGGCACACAACCTTGTAATGTGCCCAACAGCATTGTGCTGTAGAGTACGAAGG
TCCGTGCGAGTGCTATGAATGCATAGCCGTAGTGTGCTATCACAACCTGTGACA
ATATGTCACAATGTAGTAATTGCTTGTGGCTTGTCTGCTTGTCCATCTGGCC
GGTCCACACCATCTGTATCCTCCTCATCTGAGCTGTCACCTAATTGCTCATAG
CAGTGTAGGTCAGTTGTTTCAGGTTGCAGATCTAATATATAATCTTTTATAGT
TGCTTTGTCTCCACGCATGACGTTACACTTGGGTCACAGGTCGGGGTCTCCAA
CACTCTGAACAGCGCCCTGTCCAACGACCCATAATATTATGAAATCGCTTGTT

TGCATTAACATGTCTTTCTTTTTCTTCAGGACATAATGGCGTTTGACAAATTAT
ACATCTAATAGTTATTTCACTTAATGGTTTTTTTACCCTCTCTTCTAATGTTTTT
CCATACAGTGAATATTGATAATGCCTATATTCCTTATCTTAGATAAAAAGCG
TAGGCACATAATACACACGCCATATGGATTATTGTCTCTATATACTATTTCGTA
AATCTGTAAATAGAACTTGTATACCTCTCTTCGTTGTAGCTCTTTTTTGCCT
GCACACACTGCAGCCTTATTTTCATGCACCGATTCTTCCAGCACCTCACACAAT
TCGTGCAGGGTCCGGGGTCGTGTTGCTGGATCCTCAAACATGGCCGTGCGTTA
GCTACACTGTGTTCTATATATATACACCGGTTTCGGTCTGACCGTTTTTCGGTTA
CACCTATTTTTTTACTAGTATAAGATTATAATTTATAATTATAAAAAAAGTG
GTTGTGGGTACGGTAACCGTAACCGGTCGTGTAGTGCACACCTGGTGAGTAA
CAGTATTTAATCATGTTTTACAAGACATATTTGGCGTAGGATGTACTTGTGTA
AGCCAACTGTAAATTAGTGCATTGAAAGTTTAAATAAAATATGTGCCTAAA
AGCAGTTTAAACATGCAATGTTAGGCAATGTATTGTTTAGCTGCACTGCAG
GACCTGTGAGTCAGCAAGAAGTCAGTTTAGGCGGGACAACAAGTGTAGACCA
GTGGACTGCAGGAAACCAAGGATTAAGCAGTTTGCTGAACTTATATAAAA
ATAGTTGTTACTGTGCCAAGGACAACCAAGTTGTGCCAAGACCAACCGAAT
TCGGTTAGGATTTAAATGTTGGATAGTACAAAATGGTACAAAATGGAGAGT
ATAAGGCTAATTAGTAGTGTAACAAAGTAGGACACAGGGTAGGGCAGGGGA
CACAGGGTAGGGCAGGAGATGCAATAGATTAGGAGCAACTGTATTGTTACTC
ATGTGGGTGCAACCATAACATAAGACTACTATAAATAGTTTAGTAAATACAG
TACTTTATTAATACATACACAAAACATACATACAGGAAGTACAAATACAAC
AAACATGCACACACATACATAACATGCAAACAACACAGTACACACACAAAA
CAATTACATAACAAACAATGGTTACCTTTTAACTGTGTTTGACATACACAAC
ACATACATGACACAGACAATTACCCAACAGACAATGGTTACCTTTTAACTTT
TTCTTCTTTGTGGAGGTACGTGGGGCCGATGATGCAGGGCGTTTTAGTTTGGG
CCTAGCCTGTAGCCCTGCCTGTAACAAAACTTCCTACCTAAAGGAACTGA
TCTAAATCTGCAGAAAACTTTTCTTTTAAATCCACCTCCCAAAACATATAGTC
CTTTAAAGGATCTTCCTTTCTTTAGGTGGTGTGTTTTTTTGACAAGTTATAGC
AGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATGCAGACGGTGGTGGG
GTAAGGCCAAATTGCCAGTCCTCTAAATAGTGGCATCCATCTTATGAATGTA
TGTCATAACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGTA
AATCAAATTCCTCGCCATGACGAAGGTATTCCTTAAATTTTCATTTTTATAT
GTGCTTTCCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCTACGAGTGGT
ATCCACAAGTGTGACAAACAAGTATTGCCCCAACATATGCCATTATTGTGGC
CCTGCGCACGTTGTAACCAGTACGGTTTATTAAATAATTGGGATTCTGAGGTT
ACCATAGAACCCTAGGAGTAGGAAAAAAGCACTGCTTTGTACAGTGGCAG
TATTGCCAGAGTTAGACCCTTGTATATATAAATCACCTGGCACAGGGTCACCT
AAGGTACCGGCCCTATTAAGGAGTGTCTAACAAACATTTGCTCACGTCTAA
GAAAAAGAACAACACTGTCACCATATGGCTCGCTAGCCATTTGCAAATAATC
TGGATACTTACATACACTGCTACATATATCAATGGGCACATCACTTTTACTAG
CTTGCAAGGTATTAATAATCCATGCAACCAATCCTGTATCTACCATGTCCCA
TCCTGTATTACACTGTTAATGAGCTGTAGGGGAGGACAATCCCCAGGATTTCC
TGAATTATTATTACAAGGGGTTCCCTTACCCAATGTTTACCTATAGGAGGTT
TGCATCCTAAAATGCATAACTGAGTCTGCTTATAATCCATAGATAAACATTCC
CTATTATCTATACCAGGTTTACCAGCATATTTGTTACTGGTTTCAGTATCATCA
AAGTTGTTTAAATAAGGATGCCCACTAATACCCACACCTAAAGGCTGTCCCT

ACCAATTTCCAAGCCTGTACAGGCCCACACCAACCTTTGGGTTTCTGGGTTAT
AAAAAGATGTATCTGGAAAACCAAATTTATTAGGGTCCGGCAATTTAATTCT
AAATACCCTGTATTGCAGGCCAGACACCTTGGGAATAAACTTTTTTACCAT
TACCACTACTGGTGTTTTTAATAGAAAAATAGGGATGTCCTACTGTAGTAAT
CGAGAACTGCCTGCATAATAATAGATGCTTGTGCGAGACACATACTCATCAG
TGCTTACAACCTTAGAGACAGGTACAGGAGGCAGGTACACAGTGGCCTCACT
AGGCCGCCACACGGACATC

FIG. 4**HPV56 Deletion Probe Template (SEQ ID NO: 107)**

CTGCAAAAAAATAGGGAATACGTTTACGGCGCCTACGTCTAAAAAATACAC
 AGGCCATAATGCAAAGGAGGATCCCTGTATATATACATCATGGGTAACATCA
 TAAGGAGACTGAGGAACAAAGGGCCACGTACTGGGGCCTGTAGGCAACACT
 ATGTCAGGACCTGAATAAAATGGTGTTCACACATTACCTAAAGGGGCAG
 TTACATTAGTGGTGTTACTAGCAAAAGACAATGTGGAAGGCTTTATAGGTAA
 GTGTGCAGAAGGTGTAGCAACTGACTGGCTAGACAAACCAGGTGCTTCATCA
 TCTATATTTGCATAAATATCATATAGGCCATCAAATGAATTATTTGCAGACAA
 TAATGGCTGCATTTCAATTTCCCTCAGCCTGTGCAATAGGACTTATATCATAAT
 AATAATGCACACGGGCACCTATTTGTGTGCCTCTACGTGTTTGTATAGTAGCC
 TTTCTGCCAAGCCTACTAAAACGTACACCACCCCTACGTGTAGTAAATGCAG
 GCCTATGTAATGCTACTATATTCATAAAATCAGGGTCAGGAGCCACACCCGA
 CGGAGAAAAAGCTAAAGATGTGTGAGTACCTTCAAAAAGTGGATTATCAGCA
 GATACTAATGTTGCAGGTCTATCAAGAAATGCAGGGTCAGTTACCTTAACCT
 GCTGAAATGCTTTTCTATATAATCTAGGAGCTGCAATACGCCTAAAGCCTGGA
 ATAGGAGTACTACTAATAGGTTCTGTACCAGAACCGTGAACAGCAAATGTTT
 GCATAGGTATTTCTTCATAGCTATGTATACCAGATGTGGGTGTGCTAATTAAA
 ATATTGCCAGACACCTCGCCTGTTTGTGGGGCCTCAATAACAGGGGGATCAA
 TAAATAACGGATTGGTTATATGGGTACTACTGACATGTACAGTACTAGAGGT
 TGGTGTAAATATCCAACACGGCAGGTGTAGTTGTTGATGAGGATGTAATTTCA
 AATCCCCCAGACCCAGTAAAATTAGGAATCCCTGCACCAGATTCTATAACAC
 TGGACTCCTCAACTAATGTAACAATGGAAGGGTCTGTAGGCCCTACGGATTTC
 CACAACAATAGGTGGTCGCGCCGGAGTTACATCAACTATTGTGGAAGGCCTA
 GACCCCAATGGAACATAGCCTGCACGACCCCCAGACCCAGTTCCCTGTACCAA
 TGCCAAGGCCTCCAAAATATGTAAATAAACTTCCCCATTGCAATATTTTATCA
 GCCCATGTTTTTTGCTCTATTTTATTAACAACATCCTCTGGACATGTACCAGAC
 AACTTACATGTTTTATATAGTTGTGTTGCAGATGCGCGTTTTCGTCGTGTGGC
 ACGGTGGGCAACCATAGTAGTAACAGTACTACTACATTTACATATTATTTATT
 ATCCACAAAGCATGTAAATATATACACAGCACAGGACAGTAAAAAATATCA
 AAAATATTATAAATGTATTAAAAAAGGATGTGGCTATAACAAACCAAAACAA
 TATTATTAATAATATACAACCTGGAAAACACAGAGGCAGATAGCAAAAGCGG
 GACAACATGACACACAAGCAAACAAACACAAAAGCAAAGCGCAAACACACC
 AATAACACAAAAATCAATGGTTGCAATATATATGCGTAGCAGCAATGTATGG
 CAGGTACACTTCACGTATTTGTGTGTTACAATAACACAAATGTATATACAATA
 TATACAGCACTTTATGGAAAACCTATTTGTCCCAAACCTGTACACTACT
 GGAATTTTTTACATGACTTAAAAAGCTGTTTCGTTGTGTTTCATCCTTATATAT
 AATTGTAATTATGCTATAATTTTTATTGTCTGTACTTGTCCAATGATATGTTGA
 TGTTACATCCACAAACAATGTTTTATATTTTGAATCGATATCTACAACATT
 TTAATCTGTTAGGTTACACCTTTAAATGTACTACAGGCGTAGTCTTATACCA
 GGGTGGTTGTTGTTGTTGATACTTCTACTTCTACTGTGCGTATTGTCTGTGTGCG
 CTGATGTGTGTGTGTGTTGTGACACACTTTGCGTGGGACTCTCTGGAGGAGTC
 AAATTCTGATTCCCGTAGTCTGGGTGCTTTTCCTGGTCTGTG**GGATACTGCC**
GCGTCTTGGTTGCCACGGacgtggaggtggtggtggtggtgcttgggtgtgtattcgtaacagttcaa

TGTTGAATTGTGGCTCCATGGATATGTCCACACAGAATAAGCTGCCTTTTATA
TGTACCGTTTTTCGGTCTTAAACCCTTTTCGGTCACTCCCAATATATAAAAGTA
TGATTGAACTTTCAACAATTAAGAAACCTGTTTTTGCACGACCGTAAAC
GGTTTTAAAAAATGAGTAATTGTATGTTACATGTTTGCAAAACAGATACTGAC
AGATACTTGGCCTACATAGTGTATTCTGCAAGCCAAAACACCAAAGTTGCAG
ACACATGTATAAAAAATGTGTGCCCAACAGCACTAAAGTATGCAGATAAAAGC
ATAGTTGGCAGTCATAGTACCTGTGAGTAATACAGGGTGCGGTACTGTACAT
AATTCAAGTATAAAACACAGGAAACGGCAATATGCCAAAGGATTAAGTGTTC
TGCTGAAAAGGGCCTTTTGGTTTTAAATAATGGCACTAGGCCATGCAACCGAA
TTCCTTTTAGTTTTAAATAATGGCACTAGGCCATGCAACCGAATTCGGTTGCAT
GTACAAAATGGCGGAAAAACAACAAAATGGAGTATGTTATGGTATATATATG
TATGCACAATACACATAGTACACTGTACACAATAACTTACAAAACAAAAGCC
ACAATAATGACACACCTTAGTTTATGCAACCACGCGTAAAAGCACTCATTCA
TGTTTATTACAGACACACAAACATTACACATATATACAGTCATACATAAATG
AATACACACACAAACATTTACACACAAACACAACATACACATACAAAGTATAA
AATAAAGCACATACACAAACATACCATATATACAACACACAAACACAGTTAC
ACAAACACACAACAACACACTACCGCCTTTTACGTTTTGCTGGTGTAGAGGT
GGAGGTAGGAGCAGATCGCTTTTTAGAGGTAGCTACAGCAGGCTTTGACCTA
GTGCCAGTTGCATTAAAAATTTTCTACCCAGTGGAAATTGATCCAGGTCTGT
AGAAAACTGTCCTGTAAGTTAACATCCCAAAATTTATATtagctaagtggctcgtgtt
ttctgttggtggctgttccgttgacatgttatagctgtgcttcaacatatctatatttctctaggctgggtggccactggcggggat
aaccaatattccagctccagtaggtagcattcatattatgtaaatatgccataacctctgcagacaaagtaatttgcataattga
aaaacaaatTGTAAATTCATATTCCTCCACATGTCTAAGGTACTGATTAATTTTTC
GTGCATCATATTTACTTAACTGTTCTGTAGCAGTACTAATAGTCATGTTAGTA
CTTCTAGTAGTATCTACTACAGTAACAAATAATTGATTACCCAGCAAATGCC
ATTATTATGGCCTTGGGCACGTTGCAACCAATAAGGTTTATTAAATAACTGTG
CCTCAGACGTAATCATAGACCCACTAGGCGTAGCAACATATACAGAACTCGG
AGGGGGTTCTCTACCATTGCTACCCTTTAAATATAACTCTGCAGGTATTGTTT
CCCCAACTTTACCAGCCCTATTAAAATAATGTCTGGCAAATAATTGTTCCCTG
CGTAAGTAAAACCATAGAAATCACCATAGGCATCTGCAGACATTTTTTAAAT
AGTCAGGATATTTACAGGTGGATTGTACAATGTCTAAAGGTACCTCAGCCTTA
GATTCCTGCAACACCTTAAAGTCCATAGCGCCAAATCCTGTGTCTATCATGTC
CCCATCCTCTATAGGTGTATTAATTAATGCAAGAGGCGGGCAGTCCCCCTGTGG
TAACTTGTGTGGACTTACACACAGCACCTTTAGTCCAATGTTACCCATAGCG
GGAGTACATCCAACAATACACAACTGTGTTTGCTTGCCATCAACTGATATATT
GTCCCTACTATCTTCTATAACATTATTATTTGCTAAATTGGAACCTTTCAGTATC
ATCCAGCCTATTAAACAATGGATGGCCACTTAGCCCAGCACCTAAAGGCTGT
CCGCGGCCTACCTCCAAACCTACACATGCCCACACTAACCGTTCCTGGTCCGG
ATTATAAATATTAGTATCTGGAAGCCCAAACCTTATTAGGGTCGGGCAACCGT
ACCCTAAATACCCTATATTGATATGCACTAACTTTGGGAATGTTTGTTTTGGT
ATTGTCCTTAGTCACAGAGTAATAGGGATGTCCTACGGCAAGCAATCGTGAA
CTGCCTGCATGATAAAATATACTAGTGCCTTTTACATAGGAATCCGTTGCCAC
AACCTTTGAAACAGGTGTTGGAGGTAGATACACCTTATTTTCACTAGGCCGCC
ACGTCGCCATC

FIG. 5

HPV58 Deletion Probe Template (SEQ ID NO: 108)

TCTGCAAAAAAATATGGAAAACGTTTACGTCTGCGACGCAAAATAAAATAGC
TAGGGTGCAACATAAAATCAGCACCATCCACAATTATGGTATTAAAAGGAGT
TAGTGGAGATATAGGAATAAATGGACTAGACATAGATGTTACAGAAGATGCA
ATGTCTGGACCAGGTTCCAATGACACAAGAGGAGTGTCAAATCCAGTATTTA
ATGGTATGGACACATTACTGGTACGTGTGGTGGCAAAGGACGTATGTGAGTG
CAGAGGACTCTGAAAATCATGTATAGTATCAGCATCGTCAGCATAAATATCA
TAAAGTCCATCATTAATACTATAGGGAGAAACAGAAGTATTTAAAGATTGTA
ATTCAAATTGTTGCTGCTGTTGTACCTGTTCTGGACAGGCTGTATGGGACTT
AAGTCTTGGTAGTAATGTACTTTAGCCCCTATTTGCTTTCCACTGCGAGTACG
AAGTGTAGCCTTTTGCCCAACCCTACTATAACGTACAGTACCCCTGCGAGAG
GTTAATGCAGGTCTGTGTAATGCAACAATATCTAGAAAATCAGGATCAGGAG
CAGGCGATATGTCACTATGTTGAAACTGCAATGTGTCTCAGGGTTAAAGCCT
TCAAATGCTGGATTATCATATGTTACAAGTCTATGAGGAGATGTTAAAAAAG
CAGGGTCAACAACCTTAACTTGTGGGTGTTGCGACTGTATAAACCAAGGCG
TGCCACAGGGCGAGACCCTGGAATGGGTGTGCTAGACGTGACATTGCCACTG
TCAGTAGAAATAACAAAGGTATCCATTGGTATGTTTTTCATAACTATGTGTGCT
AACAGTAGGAGAGGAAAATATTAAATGTCCAGAGGCCTCTGCAGGTGCAGG
AGGGCGGAGTACGGATGGCTCAGTAAAGGAGGGATTAAATGTGTAGAAAC
AGTTTGTATAGATGATTCTCCAATAGAGGAAACATTAAGTATTGCAGGTGTA
GTATCTGCAGAGGTGGTAATATCAAAACCAGATGGAGTGGGAATTGATGGGG
CTGGTGCACCGGCGTCTATAAACTAGATTCTCTATTAAAGATACAATAGA
AGAATCCAAAGGCCCCACAGTATCAACGGTAACTGGGGGACGTATGGGCTGT
AAAGGTATAGCCTCAGACGGTGGGGTACTACCAAGGGGCACATATCCAGTCC
TGCCACCTGTACCCGACCCTGTACCAATGCCTAAACCTCCAAAAAACACCCC
TAAGCTACCATATCGTAATATTTGATCTGCTATAGTAGTGCCTTCAACTTTGG
GTATAACATCAGGTGGGCAGGTGCCTGAGGCCTTGCATGTTTGGTAAAGTTG
TGTAACAGATGCACGCTTGCGCCTTGTAGACCGTTTGTGTCTCATAATAAGAC
AGTGCTAAAAATATAAAAGTATTTATTAGTAAATGTATAAAAAATAAAAA
CAACACACACAACAGTAAATATTTACAATACCATAACCACCATGTGCAGAACC
AGTATACAGTTAGTCTTGTTGGGTAAAGTATTGTGCATGAAAATTAATACACA
TCATTGGTATATATAAAAAATATTAAGTAACAGAAAAAAATTCGTAGAGCCGA
CCCCACAGACACCCAAAGCAGCAACACCAACACCAGCAACCAAGCATATAT
AGAAATAGATAGCACCAATGGCCGCAAAAAAATGCACAAGCATAAAAAACAG
TATAAAACAAACAACAAAAATAGGTAATATCATTGTATATACACTTAGCAGC
ACATATTGGCTTGTGGTTTACATACAGTAATTGTACAATACAATTACAATGAC
ATAACACCAGTACTTATTTGCACAGTGGGTGGTATTTTAACAGTGTTTAAAAA
CAGTTGTCGTTGTGTTTCCGTTGTGTATGTTACAGTAACAATTCCTACTTTGTC
ACTTTGTGCATCACTGGTCCAATGCCATGTGGATGACATATTACAGTATAAGT
CTTTAAATGGTTTTAATCTATATCTTAAACATTTTAAACTATTTGGGTACCTT
TTAAATGCACGATAGGTGAAACTTTAGA ACTACACACGTTCCGCCCTTTGTAT
GTACAGTTAGTTGTACTGTGTAGTCCTCCTCGTGGTCTACTGTCCACGGC

GCAGTCTGTATACTTTGTGGAGTACTGGGTGTTGTCTCTGGAGTCTGGTAAAT
CGAGTCGTCGTCGCTTTGTCCCCTGTGTACTTTTCGTTGTTGGTGGCCTCGGTGG
TCTTTGGGTCAGCAGTTTCAGTAGTGGATATTTGATCACTAGGTATAGATGTA
GGACATACAATTACCCGACTACCCACATGTACCTCCCATAATTGTGTTTTAGA
GTACTTTTTTGCATCCTCTTTAAAATATTTAAAATACGTCTTTTCATTGCCATG
TATATAATACAACCCACATAGTCAACTTCTCCTGCTACCAAAGTACATGTTG
TTTCCTCAATAATATATATTTCACTCCAATTTGTATAATCCATTGTGTTTGCTT
TATCATTGTCATATTGTACAGTTACTGTTATGCCTTTTTTTTTTAAAGCATTTTT
GTGGCTCTGATAACCACACTTCTAAGCTTGTTTGTGCAATGTCCATTCATCT
GTTTTATATGGTGATGCATTTAATGTCTCTAATGCCATTTGCAGTTCAATTACT
TGAAACGCTTTAGTCTTTGATGCTACCAATGACGGCACCACCTGGTGGCACA
AATGTGATATTCCCATTTGTCTGGCTGTATACATTATAGCACACTCCATGCGT
ATTAGTTTCCAATGTTCAATttgtgatgttaaattcattttatcagcttcgtatatgtctaggatttgcctgcact
gcacttaaacgtgctgatatttctccatcgtttctgtctctctctctattaagcctaatttgcaccacgtccttgagaaaaaggat
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aaatatggccatcgtaacttttgcctgcatttgtatttgaggttaatttaattgggtgacattttaattgtactaatgcctatgtttaca
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ttgtatcatttcaacttaaatcaatatatcatcattaaagctatgctgtaacactgttaactatctatccattctgggtgtgccttgcac
atcattatatttgacattgctgttctaaccataaagcacatgcttgacttcgtaatttgggtggctcgataatcatacatgtttca
ggaattgatagtaaattactcattatttggccacagttaactctatttgcacatttaaatctaattaacaataataataaattcctctgt
cacacgttaaactgttaggtgtgtatataactgtgctgtttaaattagctctttaaactttctgctacggaggactattccataccct
gttatcaccaatctgtacagctgttttatcacttttAAATGGTCTAACTAATTCCATAAACTTAC
TCCATAAGCTTCTTTGAATTTATATAATAGCGTTGCTTTAGTATTACTGTTATG
TAGAATATTACTAATATTTTGAATGGAACAGTATTACAACGTGTCTACATCCG
TTTCACTGCTTACATCTGAACTAGCCCCACCCCACTAGACTCCGAGTCATTT
AAGTCTGCGTCGCCATTTTGGCTTTCTACCTGGTGTGCCATCTGCTCAGTTTCC
ACTTCAGTATTGCCATATCCGCTGTCTTCTAGCTCAATAATTTTTTCGTTTTCTG
TGTGTGCATTCTTTATTTTTATATTTCCACGATACACACACATTTGCAGCCCGG
TCCACACAGTCCTCTACAGCACTTTCTGAGCATGCTGCAAACCTTTCGTTTTAG
TGCACACACAGCATTTATATCGTCCACCCCTTCCTGTACATTAAACAACGCTC
GGGCTGCCTCTGCTTCTGCCTGTGTAGTACTTTGTACTGAATCATCTATAAAC
TCTATTAAATCTGTACCACTATCGTCTGCTGTTTCGTCCCTCATCATCTGAAATA
TTATCTCCTGTTCTTCGTTCTATTACCGCTTCTACCTCAAACCAGCCAGTACAG
CCCGCCCCCTACCCCGTTTGTACCTTCAGGGTCATCCATTGCAGATGGTGTTTA
TTGCTGTGCACAGCTAGGGCACACAATGGTACATGTGCCCATAAGCAGCTGC
TGTAAGGGTTCGTACGTCGGTTGTTGTACTGTTGATACACAAACGAACCGTGGT
GCCACAAGTGTAACAACAAGTTACAATGTAGTAATTAGCTGTGGCCGGTTGT
GCTTGTCCATCTGGCCCGTCCAAGCCTATTTTCATCCTCGTCTGAGCTGTCACA
TAATTGCTCATAGCAGAATAGGTCAGTTGGTTCAGGATGTAAATCTAAAATA
TATTCTCTTAGCGTTGGGTTGTTTCCTCTCATGGCGTTGTTACAGGTTACACTT
GTGTTTGTCTACGTCGGGGTCTCCAACACACTGCACAGCGCCCTGTCCAACGA

CCCGAAATATTATGAAACCTTTTGTTTAAATCCACATGCCTTTTTTTTTCTTGT
GGACACAATGGTCTTTGACAAATAATACATCTAATTAATATTTCATTAAACA
CTTTTTTAGTGTTTGTCTAATGTGTCTCCATATAGCGAATAATTATAATGTCT
ATACTCACTTATTTTAGATAGCAATCGTAAGCACACTTTACATACTGCAAATG
GATTTCCATCTCTATACACTATTCTTAAATCTGCAAATACAAAGTCATATACC
TCAGATCGCTGCAAAGTCTTTTTGCATTCAACGCATTTCAATTTCGATTTTCATG
CACAGATGTCTCCAACGCCTGACACAAATCATGCAATGTCCGTGGTTTCTCCT
CTGCGTCCTGGAACATAGTCCTGCAGTAGCCTACCAAAAAATGTCTGCTTTAT
ATATGCACCGGTTTTCGGTCAGACCGTTTTCGGTTACACCCTAGTTTTTACAAG
ATTTGGCATTATAGTTTAGTATTATATAAAATGTTGAAACATGAACAATGTGA
CCAAAACGGTTAGTCCACACCTGGCAATAAATGTTAGTGAGTCACTTTTACA
AGACATAGTTTGGCATAACAACTATTGTGCAAGCCAAAACCTGCACTTAAGC
ATTGAAAGTAAGTTAAAATATGTGCCTATAAGCATGTTTAAACTATGAGTAT
AATTAAACATTTTGCAACTGCACTGCATGTATATATGAGTCACATATGCAAGT
ATAGGCAAAAGCAGTGCAGGAAGGGAAAGGATTAAGTGTATTGTTTAAATTG
TAGTTTAAAAAAAACACGTTTGTGCCAGCAACCGAAATCGGTTACATGCACA
AAATGGAGGTAAAGTAAAATGGAGGCAGTACTGTTAAAACTATAAAATAC
ATATCATACAAATACTATTACATAGGTATGCATAATAGGCAGGGCAGGGTAG
GGCAATTTAGGGACAGCACCTTACTCATAGATACACCCAAACAATACAAATA
GTTTACAATACACACAATAGTTTATTACATATATACAGAAACAGGAAACTGA
CAAGGACATAGAACATGTACACAAACATGACACATAACACATACAACATATA
CACAAACATAAAACAAACAGACAACACATACATAAAACAAACAAACATGTAT
AATAAAATAGTGTAAGTACCACAACAATTATTTTTTAACCTTTTTGCGTTTGG
TGGATGGTGCACGGGTAGTAGGGGCCGAACGTTTTAGTCTGGGCTTTGCTTTA
AGGCCTGATTGTAATAAAAACCTTTCGTCCCAAAGGAAACTGATCTAGATCCC
CCTCGACTCTAGAGGATCTGCAGAAAACCTTTTCCTTTAAGTTAACCTCCCAAA
AAGTATATTTATTTAATGGATCTTCCTTTTCTTTAGGGGGTGCTGTTTTTTGGC
AAGTAATAGCCTGGGAGGTAACAAATCTATATGTGTCCTGTAAACTGGCAGA
CGGAGGAGGTGTTAAACCAAATTGCCAGTCCTCCAAAATATTGGAATCCATA
GTATGTATATATGTCATTATCTCTGCAGTTAGTGTAATTTTGCAAAGCTGAAA
AACAAACTGTAAGTCATATTCTTCAACATGACGTACATATTCCTTAAAATTAT
CATTTTTATATGTACCTTCCTTAGTTACTTCAGTGCATAATGTCATATTAGTGC
TACGAGTGGTATCAACCACGGTAACAAATAACTGATTGCCCCAGCAAATGCC
ATTGTTATGACCTTGTGCACGCTGTAGCCAATAAGGCTTATTAAATAATTGTG
ATTCTGAGGTAACCTATAGAGCCACTAGGAGTTGGAAAAAATGCACTACTTTG
GATAACTGCAGTATTACCGGACCCTTTAATATAAAGGTCATCCGGGACAGCC
TCGCCAAGTTTTCCAGCCCTATTAAAAAAGTGTCTAACAAACATCTGCTCACG
TCTAAGAAAAAAGAACAACACTATCCCCATAAGGTTCACTGGCCATTTTTAAA
TAATCTGGATATTTGCATGTACTGTTACAAATATCAATAGGCACATCACTTTT
ATTAGCCTGCAATGTACCAAAGTCCATGCATCCAAACCCTGTATCTACCATGT
CACCATCCTCAATAATAGAATTA AAAAGTTCCAATGGAGGACAATCAGTAGC
AGCTGCATTATTGTTACAGGCAACACCTTTACCCCAATGCTCACCAGTGGGAG
GTTTACAGCCAATTAACATAATTGTGTTTGTGTTTATAATCCATAGATAAGCAT
TCCCTGTTATCAGACCCTGGCTGTGCGGGATATCTGTTACTGGTTTCAGTGTG
ATCAAATTTATTTAAATAAGGATGACCACTTACGCCAACACCCCAATGGCTGTG
CCCTACCTATTTCAAGGCCTACACATGCCCAGACCAACGTTGTGTATCAGG

GTTATAAAAAGATGTATCAGGAAAACCAAATTTATTGGGATCAGGTAAACGC
ACCCTAAAGACCCTATACTGTAAGCCTGATACCTTGGGAACTAATACTTTTT
ATTGTTATTGGGACTTTTGATGGAAAAATATGGATTGCCAACAGCCAAAAGT
CTGGAACTGCCAGCATAATAATAAATGCTTGTGCGTGACACATATTCATCAGT
GCTTACAACCTTAGACACAGGCACAGGAGGCAGGTACACAGTGGCCTCACTA
GGCCGCCACACGGACATC

FIG. 6**HPV66 Deletion Probe Template (SEQ ID NO: 109)**

CTGTAAAAAATAGGGAACACGTTTACGGCGCCTACGTTTAAAAAATATAC
AGGCCATAGTGCAAATGTACCTCCCTGTATATATACATCATGTGTAACATCAT
AAGGAGACTGAGGTACAAAGGGCCAAGTACTGGGGCCTGTAGGTAAAACTA
TATCAGGACCAGAATAAAATGGTGTTCCTCAAACATTTCCCAAAGGGGCAGT
AACATTAGTTGTGTTACTAGCAAAGGATAATGTAGAAGGTTTAATAGGTAAT
TGTGCAGAAGGTGTAGCACCAGACTGACGAAATGAAATGGGTGCCTCATCAT
CAATATTTGCATAAATATCATATAGGCCATCAAATGGATTGTCTGTAGACAAT
AATGGCTGCATTTCAATTTTCATCAGCCTGTGCAATAGGACTTATATCATAATA
ATAATGCACACGAGCACCTATTTGTGTACCCCTACGTGTTTGCATGGTAGCCT
TTTTGCCTAGCCTACTAAAACGCACACCTGTTCTACGTGTAGTAAATGCAGGC
CTATGCAATGCAACTATATCCATAAAATCAGGATCAGGAGCCACACCCGAGG
GAGAAAAGGTCAACGTTGTGTGTCAGCACCTTCAAAAACAGGATTATCAGCAGT
TATTAATGTTGTGGGGTTGTCCAAAAATGCTGGGTCAGTGACCCTAACCTGCT
GAAAAGCCCTACTATACAATCTGGGAGCAGCAAGGCGTCTAAAACCTGGAAT
AGGGGTACTACTAATAGGTTTCGTTGCCAGTACCGTGTATAGCAAATGTTTGCA
TAGGTATTTCTCATAGCTACGTATTCAGATGTAGGAGTGCTAATCAAAATA
TTACCAGATACCTCTCCAGTTTGTGGAGCCTCAATTACTGGAGGATCAATATA
TAGTGGGTTTGTATAGTAGTACTACTTACATGTACAGTACTAGATGTGGGTG
TAATATCCAACACAGCAGGTGTGGTTGTAGAAGAGGATGTGACCTCAAAGCC
CCCTGACCCAGTAAAATTGGGAACACCAGCCCCTGAGTTAATAACACTAGAT
TCTTCTACCAGTGTAACAATAGAAGGATCCGTAGGCCCAACTGACTCCACCA
CAATAGGTGGTCGTGCAGGAGTGACATCAACTATAGTAGAAGGCCTAGAGCC
TAAGGGAACATAGCCCGCCCGACCCAGCCAGCCAGTACCAATGCCA
AGTCCCCCAAAATATGTAAATAAACTTCCCCATTGTAAAATTCTATCAGCCCA
TGTTTTTTGCTCTACCTTATTAATAACATCCTCAGGACATGTACCAGGTAATTT
GCATGTTTTATATAATTGTGTGGCAGATGCGCGTTTTCGTCGTGTGGCACGGT
GGGCAACCATAGTAGCAAAAACGTAATACTGTTACAAATGGTTTATTAACCA
CAAAGCATGAAAATATATACACAGTGTAGGTATATAAAAAAATAGTAAAAA
CAGTATAAATGTATCAAAAAATGATGTAGCCACAACAAACCAAAATAAAGTA
ATTAGTATAAGACAATTGGTAAACAAAGATGCAGATAGCAAAAAGCGGGACA
AAATGGCACACACACAGACAAACACAAAAGCAAAGCGCAAACACACAAATT
ACACAAATATCAATGGTTGCAATATATAGCGATTACACGTTACCGTTTCCAGT
GTTACAATAACACATACTATACCAAATAACAATTAATGGGTGCAATATATATG
CGTAGCAGCAATGTATGGCAATTACACTTCATATGTATCCGTGTTACAATAAC
ACATATGTATATACAATATATACAACACTTTATGGACAACCTCATTTGTCCCAA
AATAACCTGTACACTAGGTGGTATTTTTACAACATTTAAAAAGGTGTCCCGTT
GCGTTTCATCTTTATATAAATATTGTAATAATACTACTGTCTTTATTATCTGTAC
TTGTCCAATGATATGTTGTTGTTACATCTGTAAATAATGTTTTATATTTTGAA
ATCTGTATCTACAACACTTTAATCTATTGGCTTCACCTTTTAAATGAATTGCA
GGTGTAGTCTTATCACCACAGTGGTTGTGTGTGTTGATACGTGCACTTCTACT
GTTGGCGTTGTTACTGATGTCTGTGTCTGTTGTGACACGGTGTGCGTAGGACT
CTCTGGAGGAGTCAGGTTCTGATTCACTTGCTCTGGGTGCTTTTCCTGGTCTGT

GGGATACCGCGGCGTCTTGGGCGCCACAGAGGTGGAGGCGGTGGTGGTGGT
CCTGTGGTTGTTGTATTCGTTAACAGTCTCAACAGGGGGTACGTTGTATCTAC
AGGTACTAGACACAGAGTCAGGACAATAAATACTCTCGGTTTCCATATGTAC
TTCCCATATGTTTGTACACCCATATTTTTTGGCCTCCTGTTCAAAGTCTGTGTA
ATATGTTTTGTGGCCATCATGCATATAATATATGCCTCTGTAATCCACCCCTG
ATGACACTTTACACCACCCACACTCTCCATTATAATATATAAATTTCCACACC
ACATATTCCATACAATTATTTTTGTTACCATCAAACCACACTTCTATATGTTT
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aaatttaactgagcatgtcctttcaaaaaacatttccaatttaccattactcaattcatacacaggattaccattgttatctaatggaa
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ggaggacattttattgtactaattgtttatgttctctatctaaacttatgggattcccatctaataaattcttagataatcatctatatact
ccaacacgtatctgttgcacatccagcaaacctaatttggcattgtctagtggctgtacacaaaagtggtcttgaattacaat
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ctacttttaAATAGTTTCTTGCAATTGGTGTGTTGGTGTTTCCATATTTGTATCTAT
ATCCATATTTACCACCGAGTGCTCACTACAGTTACTGTTTTGCGAGCCTCCAT
TTTGTGAGCTCCCGCACCCATTTCCCTTTTCGTATTCTACCTGTTGTGATGTTT
CCAATGTTTCCAATGTATTGCCGTACCCGCTGTCTTCTGATAATATTAGCCTTC
GTTTTACTTCCTCCCGGTACACAGTTTGCTGATTACTAATATCACTTAAGGGA
CTACCTATATACTTTTCGTTTTAGTTTTTGCAACGTCTGTGCATCTGCATGTGCT
GTTTGTACTTGCAATAATTGTTGAGCTGTCTCCCTGTCTTCCCTGTGTATTGTTT
ATAAGTGTATTGTCTATAAATCCATCTAAATCTGTATCTGTTTCATTCTCCTCC
TCGCTTTCATCATCTGATATTGTATCCCCGTTTTTCTTTCTACAATTGCTTCTA
CCTGAAACCATCCACAACATCCCGTCCCCTCCCCATTTGTACCTTCAGGTGAT
GCCATTGCAGTTATTTAGTTGACGCGCAGAGTGGGCACGTTACTGTTAACGCA
CCCATAAGCAGCTGTTGTACCACACGTAGCTCCTCTTTGGTACTCTGAATGTC
CAACTGCACCACAAGCTCACACTTACAACAAGGTACGTGAATTAGGTAACAC
TTATGTTGTTTCAGCTTGTCTAGCTTGCTGTGGCCGCTCCAGCAAATGGTCTATT
TCATCCTCATCCTCATCCTCTGAGCTGTCCAATTGCTCATTGCATTGTAGGTCA
ATTTAGTTTTCGCGGTGCAAGTTCTAATATAATCTCTTGCAACGTTGGTGCTTT
ACCATGCATGGTTATACTGTAGATTCTGTGGCTTGTCTACTCGTATGTCTCCA
ACACTGCAAACATGACCCGGTCCATGCATATGCTATATAATGAAATCGTCTTT
TATGTTACAGTGCAATTGTTTTTCTCCTCCGGTGTTAACGGACATTGACATCGG
TAGCACCTTATTGATAAATCAGATAACTGTTTTTTAGTTATACCTTCTAATGTT
GCCCCATACACTGAATATTTATAGTACCTATATTTTCTAACCTTACTATAAAA

CAATAAACATACCCTACATACTGCATATGGCCAATTGTTTCTATATACTAATT
TTAACTCAATACATGCAAACCTATATAACTCTAAACTTGTAAGTTCCTTTTTG
CAGTATACACATGATAATCTAAGATCAAGTAAAGGTATTTGTAATACCTCGCT
CAGATGGTGCAGGCTTCGTGGACGTTCTGTGTATTGCTGAATATGGAATCCA
TGGATATCTACAGGCACAACAGGCTGCCTTTTATATGTACCGTTTTCGGTCCT
AAACCCATTTTCGGTTACTCCCAATATAATAAAGTATGATTGAAACTTTCAACA
ATTAAGAAAGAACCTGTCTTAGCACGACCGTAAACGGTTTTTGAAAAATGAGTA
AGTATATGGTTACATATTTGCAAGACAGATACTTGGCTTATACAATAAGGGCT
ACACGCCAACAAAATATATTGCAAACACCTGCATAAAAACATATGCCCAACA
GCGCCAAAGTATGCAGATAAAAGCATAGTTGGCACAGAAATACTGGTGAGTA
ATACAGGGTAAGGCAGTGTACATACCTGAATCATACAACAGGAAACGGCAAT
ATGCCAAAGGATTAACTGTTTTGCTGAAAAGTCGTTTTGGGTTTAAATAAGGA
CAAAAGGCTAGGCAACCGAATTCGGTTGCATGCATAAAATGGCGTACAGCAC
TAAAATGGAGTTTGGTATGTTACTATAGTGCATACAAACAACCTATTTAATAT
AACAGTAAACAGTAAGGAACACCACCTAACCTGACACACACTGCCCAAGGA
TACTACCGCACCTTAGTTTATGCAACCACGCGTAAAAGTAACCATGCATGTTT
ATTACATACACACAAAACATTACACATACATACAGTCATACATACATAGC
ACATACACAATTATAAAATACATAAACATACACAAAAACATACAGTACAAGC
ACAACCATAACAATACATAACAACAGACAACACACAACACTATCGTCTTTTACGTTT
AGCTGGTAAAGAAGAGGAAGAGGTAGGAGCCGCCCGCTTTTTAGAGGCAGA
TGCACTAGCCTTGGGTCTAGGGCCTAGTTGCATTAAAAATTTTCTACCCAAAG
GAACTGATCTAGGTCTGCAGAAAAGCTGTCCTGTAAATTAACCTCCCCAAA
CTTATATTTAGCCAGGGGATCCTGCTTTTCTGCAGGGGGCTGTTCCCTTTGAC
ATGTAATAGCTGTGCTTTTAAATATGCCTATATTTATCCTCTAAGCTAGTTGCA
ACTGGTGGGGACAATCCAATGTTCCAATCGTCTAATAAAGTATTATTCATATT
ATGCAAATATGCCATAACTTCTGCAGTTAAGGTTATTTTACAAAGTTGAAACA
CAAAGTGTAGTTCATATTCCTCCACATGGCGAAGGTATTGATTGATTTCACGT
GCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAAATAGTCATGTTGGTACTT
CTGGTAGTATCCACAACAGTAACAAATACCTtgattacccagcatatgccattattatggccctg
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tgggaggagggtccctgccattgccaccctccaatacaaatctgtaggaatggcttcccaacattacctgccctattaaagtaat
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TCCATTGCACCAAACCCGGTGTCCACCATGTCACCGTCCTCTATTGGGGTATT
AACTAATGCCAAAGGTGGACAATCCCCTGTATTGACTGGTGTAGACTTACAC
ACCGCGCCCTTAGTCCAATGTTACCTAATGCTGGCGCACATCCCACAATACA
TAACTGGGTTTGTTTACAATCAACAGATATATTGTCCCGGTTATCTTCTGCAA
CATTATTACTTGCTAAATTAGAGACTTCAGTGTATCCAGCCTATTAAATAAT
GGATGACCACTTAACCCAGCACCTAAAGGTTGACCTCGGCCTACCTCCAAAC
CTACACAGGCCCATAACAAACGTTCTGGTCAGGATTATAGAAAGATGGATC
AGGAAGGCCAAACTTATTAGGATCAGGCAACCGTACCCTAAACACTCTATAC
TGATATGCACTAACTTTAGGGATGTTTTGTTTTGGTACCAGATTTAGAAACAGA
GTAATAAGGATGGCCAACAGCAAGCAACCTAGAGCTACCTGCATGATAAAAT
ATACTGGTACGTTTTACATATGTATCCGTTGCCACAACCTTTGAAACAGGTGT
TGGAGGTAGGTACACCTTATTGTCACTAGAACGCCATAGAGCCATC

FIG. 7**HPV68 Deletion Probe Template (SEQ ID NO: 110)**

CTGTAAAAAATAAGGAAGGTGTTTACGTTTTTTTAATAAAAAGAATAACAA
TGGTAATAAATAATAATTGGAACCATATATAGTTATGGCAAAGGTTGTATCA
ATTGGAGTAGAGGGTGTAAAGGGCAACTGTGGGGTGTAGATGGTAATACAA
CATCAGGACCAGTATTTACAGGCGTGTTCCAAGCAGTACCAAGAGGAATGGT
AGTATTAGTATATGTAGTGGATGCAGCAGAAGCCAATGAAGGAAGTATATG
TGGGAACGAGTAGTAAATGTAGCATTATGAAATGCAGTATCTAATACTGTAG
TATTGTCAGTATCAGGTGCATATATATCATATAAGTTATCCATAGGGTCCGCC
TGCTCAGGGGCAACCAATGGTTGTAGTTCAATGCTGTCAGCAGGGGTAAATGT
TACTAATATCATGATAATAGTGCACCTGTGCCCCAATTTGTGTACCTCGGCGT
GTAAACATGGTCGCCTTTTTGCCTACTCTGCTAAAACGTACTGTTCTCTTTCG
GGATGTTAAGGCAGGCCTATGTAAACGAACAATGTCCAGAAAATCCGGATCA
GGAGCTATGTCAGCAGGTTTCATATGTAAGAGTAGTATCAACAGGCTCAAAAG
CAGGATTATCAAATGTTACAAATGATGAAGGGTGCGTTACAAAATCAAATTT
ACTAACACGAACCTGTTGATGTGCCCTACTATATAAACGTGGCCCTGCCACA
CGACTAACCCCAAGGTATAGGAGTACTACTAATAGGTTCTGTACCAGTGCCAT
GTGTTGCAAATACCTGCATAGGTATTTCTCATATCCATGTGTTCTCTGATGTA
GGGGTACTTACAAACACATTACCAGACACCTCACCTGTTTGTGGCACTTCTAT
TATAGTCGGGTCTGTAAATGCAGGGTTAGTAAACTAGTACTGCTTACCTGCA
CAGACCCGGACGAAGGGGTAAATGTCTAAACTGCCGGTGTAGTGGTAGACGA
AGATGTAATTTCAAACCCAGAGGTGCCTGTAAATGTTGGTACTGGTGTCCCA
GATGTAATAACACTGGAATCCTCCACCAATTGCACAATGGAGGGTTCCGTAG
GACCCACAGGTTCAATAACACAGGTGGACGTGCAGGCGACACATCCACAAC
AGTATTAGGTTTTCCACCTAAAGGAATGTACCCAGCACGACCTCCGGTGCCA
GAGCCAGTACCTATACCCAGGCCACCCAAAAATATACCTAACTTGTCCATT
GTAATATTTTGTCTGCAAGTGTAGTACCTTCAACCTTATTTATAACATCAGAA
GGACATGTCCCTGATTGTTTACATGTTTTATATAAGTCAGTTGCAGATGCACG
CTTGCGCCTGGCAGCACGGTGTGAAACCATATTTATTTATACAAATAAACAA
CAATACAAATGCACACTATAACAGTTACTGTATAGTATTAAGAGTAAGACTG
TGTAATAACCACATAGGCAGTATAAAAAAAGTAGGTATACAGCAAACACCT
CAAATGGTGTAGTTCTAACTAATATAAACACAAACACAAATATCCACACATA
CACACACACATGCACAGACTGCAAAAGCGGGACAGTGCAACATATATACAT
GCATACACAAAACCACACCAAAAAATACCAATACAAGCATATGTATACAATAT
ACATATAGTGTACAGTGTGGGTCAATACACACTTATAATGTCATATACCCCAA
TGACACAGTTACACTAGATGGTAGTTTAACAGTTTCCAAAAACTTGTACAGTT
GTGCTTCTGAAACATATGTTACAGTCAATATACCTGTATTGGTTGATCCCCTA
CCCCTTATCCAATGCCATGTACATGATATATTGTCATACAAAGCGTTATGTTT
TTGCAACCTATACCTAAGACATTTTAATCCATTTTTGTACCTTTTAAATGCAC
TATAGGTGTAGTGTACCCACAACAAAGGCTCCGTCTTTTGTGTTTGGCCTGCAC
TTCTACTGAGGAGTGGGAGGTGACACCGTCCACGGACACGTTGTGCGGGCTC
AGAGGGCTCAGTGATTCCGCACTGCTCTGGGATACTTCCGAGACGACGTCga
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tctattccatgttgattatgttaacttttatccattccggcgtgtgccacacacctactaataattagatatctctgtctatacaca
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ctactgttattctatttttccacattgtatcttattagcattagtatttaattccagttttgtatctaaacattgtatatgggtatacaatg
catattgtttaattagtgtttaaaccttcgcaatgggttgattactccaaatattgctgtaccagctgtacatgttggtttatcac
tttaaatGTACGTACCAGGTCATTAAATGACAATCCATATACTTTTTTAAATTCT
GTTAACATTGCAGCCTTTTTATTGTTACATTGTAATAATACTTTAAGTTGGGTA
GTAGGTGATTTAGGATCCTGGTTTTCACTATCTATAGCACTGTCCACACTACT
ACAGTCCTCCCGTATACTGTCGCCATTTTCCCCTTCATTTTCCCCATCCTCCCC
ATTTATATTAGGTGCTACAGTTACCTCCGAGTTAGTTTCCACTTCCATATTGCC
ATAGCCGCTGTCCGGCACTGTATACGCCGGTTGTCTTGCCTGTGTACTGCTTA
CATTTAGTGATAATTCCTGTAATGGCGACTTTGCTAAAGGACTGCTTTCTATA
CTGTCTGTATACTTTCGTTTTAGGGCACGCACCCTTTGTGCATCCCTTTGGGCC
TCTTGCATATTCAAAAGTACCTGTGCTGTCTCACGCTCTGCCTGTATACAAAT
ATGTGTAGAATCATCAATAAAATCTACCATGTCTGAACCTGTATCTGTTGCGT
TTTCGTCCTCATCCTCTGAGACTGTGTACCTGTTTGTTTATCTACTATTGCTT
CTACAAAAAACCATCCGTTACACCCCGTCCCGTCCCCATCGGTACCTTCACAA
TTGGCCATTGCAGATTACTGGGTTTCAGTTGCACACCACGGACACACAAAATT
TAGTGAGTCCATAAACAGCTGTTGTAGTGTCCGCAGGTTGTCCCGCGACGCTT
CTACTACTAGTTGCAGTGCCTTGTTACACTTACAACACAGACACTGAATTCGA
ATTCTGTGACGCTGTTGTTTCGTCCTGCTGGCTAGTAGTAGATGTTGGTGGTG
ATTAACCTGCATGGTTCGGGTTTATCTATTTTCATCGTCTGAATCTCCTAATTGCTC
GTGACATACAAGGTCAACCGGCTGTATTTCAATTGTATGGACATAGCTCTAACA
CAATTTCCCTGCACGGTGGGCTTTGGTCCATGCATAGTTACTTAAACTTGTGTT
TCTTGACGTATGCGTCTGCGGTCTCTCGCTTACTGGTCCAGCAGTGCCGACA
CTGTCCTGTAAAGTTTCCTGCTATTTTATGTAATCTTCGTTTTGTTGTTAGGTG
CCTTAGTTTTTCTGCTGGACACAATGGTTTCAGGCAACTCATGCACCTTATCA
ATAAATTATATACTTTGTATTAGTTATGGTTTCTAATGTAGTTGCATACACC
GATTCCGAGTAATATCGTAGTTCCCGTATTTTAGCATAAAATTTAATACATGA
TTGGCATGCAGCAAATGGTACCCCGTCTCTATACACTACACATAGGTCATAA
AGGCAAATTCATATACCTCTGTCCGTTGTAGTTGCCTTCTGCAATAGACACAG

TCTATTGTAACGTCATGCAATGTAGTGTCCAATGTCCTGCACAGGTCTGGCAA
TTTGTATGGCCGTTCCCTCAGGGTTGTGAAATAGCGCCATTAGTATAGAGAACT
GCTGTGTTTCAGCTTTATATACACCGTTTTTCGGTCGTGACCGTTTTTCGGTCCCAC
CCTTTTTTTATATAGAATAATTGTAAAATGTTATAAGTAAAAGTATTGGTATG
TGTGCCCAACCTTTTTTCGGTTGCACAAATTTATGGATGTAGTATTAGTCACTG
TATAACTTACACTACCAGACAGATTCTTGAATGCAAAAGTAGTTATACTGGCC
AACTATGCTATTAGGCAAGCAAAAACAGTTAAAACCTTTATGCCTAAAAGCAG
TTTTATTACAAGGGAGGAGATACGTTGGTTGCCAAACTATTGTTGCAGTGCAC
CTGGACAGGATGATGACTAACTAAGGTGCGCCAGTACGACTGTTACTGGTGC
CAACTATGTGGGTAACTCAAGGTAAAACATGTTTTTGTGAAACACATAGGT
TTTAATACTATCTAAAAGCAAATAGTACCAACGACCGAAAGCGGTGCGACAC
AACCCGGCCATACAAAATGGCCACACGGTATAGTTTGCAACCATAAATATAT
TGCATATAATGTAACACACCTTAGGGTAGGGCTACAAATTATGTATGAAATG
TTGCAGTCCTATTATATGTAAAACAAGGACATATGTTAGTCAAGGGTGCAA
CCACAAAGGACACGGACATACATACTTTATTAACAAAAACAGATTATACACA
CATACTTGCAAACATACACATATACACATACACATACATGCACATACACAAC
ATACCAACACCAACATGACACATATACAACCACACAGACAAACAACATACA
AAACAAGACATATAACAATTATTTTGACACACGTTTACGTTTGTGTTTAGAGG
TAGATGTGGTAGCTGCAGTGGCAGTGCCTTACGAGGGCCTATGGTGGGCCG
TCTGCGAACACCTGCCTGTAAACAGAAATTTGCGTCCTAATGGGAATTGGTCCA
GTTTCAGAACTAACTTTTCCTTTAAATCCACATTCCAAAAGTTAAGACCATCA
TAGGGATCTTTTTTAACAGGTGCAGGGGCGTCCTTTTGACATGTAATTGCTGC
TGATTGTAGGTAGCGGTATGTATCTACAAGACTAGCAGATGGTGGAGGGGCA
ACACCAAAATTCCAATCATCCAAAATAGCAGGATTCATAGTATGTATATATG
ACATTACATCAGTGGATAATGTTATAGTACACAACCTGAAATATAAACTGCAA
ATCATATTCCTCAACATGCCTAACATATTCCTTAAATTTATTAGAATCATACA
CAGCTGGTACAGTAGAGTCTGTAGTAGTGGACAATGTAAAATTAGTACTGCG
CGTTGTATCCACAACGGTAAGAAATAATTGATTATGCCAACAAATACCATTG
TTGTGTCCCTGTGCCTTGTGCAGCCAATAGGGCTTGTTAAATAACTGGGAGTC
AGAGGACACCATAGACCCGCTAGGCGAGGGGGCATAACATAACTACTAGG
AGTTTCACGAATGTCAGTGCCCTTAATATACATGTCAGTGGGAATAGTGTCCC
CTACCATGCCTCCCCTATTCCAAAAATGcctggcaataactgttccctacgtaaacaaaaaaca
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gttcttgtaatgtacaaagtccatagcaccatatcctgtatcaatcatatcgccatcctcaataggagtatttaccattccaatgg
gggacagtcccttgtgtacattggtaggcttacaagatttaccttggcccagtgctcgccaatagcaggaacacagcctataat
acacagctgtgtttgttacagtccactgcaacattgtccctactgtcttttaggattttattagaggaaaacggggaattttcagtatc
atccagcctattatagtggtatgcccactaaggccaacgcccattggctgccccctacctATTTCAACACCAAC
ACAGGCCCATACCATGCGCTGTGTATCTGGATTATATAATGTAGACTCAGGA
ACACTAAATTTATTAGGATCAGGTAAGGTAACCCTAAACACTCTGTATTGATA
TGCAGACACCTTAGGAATGCCCTGCTTGCGGCCCCCAGACATAGGAACCTTA
AAATATGGATGGCCTACAGTTAATAACCTAGATGTACCAGCATAGTAATACA
TGCCAGTGCCTGTACATAATCATCTGTATTGACAACCTTCGCCACTGAGGGG
GGAGGCAAATACACCATGTTGTCGCTAGCTCGCCACAATGCCAT

FIG. 8**HPV26 Deletion Probe Template (SEQ ID NO: 116)**

CTGAAAAAAAAATAAGGCATACGTTTACGGCGTTTATGGATTAAATAAATATA
GGGCCATAAATAAATAATTATCCCCATGCACAACCTATTGCAGGTAAGTTAGTG
GTGGGTGGGGGGGGCAATGATGGCCATGTCTGGGGAAGATGTGGGCGTGTAA
ATGTCTGACCCACTATATACAGGTAGTTCAAATGAGGTGGATAAAGGCACAG
TAACATTGGTAGTGGAAGTATTAATAGAGGAAAACACATTGGAGGCATATCT
TGGAACCTGGTAATGTTGTAGGGGAATATGACATGCGCGGCGTATGTATGCTA
GGAACCTGTATCAGGGTCTGCATATATATCAAACAAAGGTGCAGATGAATGGG
TAGATGTATGTAAAGGCTGCAATTCAATTTCTTCGTGTTTCAGCAAAAGACTGT
ATAGGACTAATATCATGATAATAATGTACTGTAGCTCCAATTTGTTTTCCACT
ACGTGTTTTTCATAGTGCCCTTTTGACCCAACCTACTATAGCGTACAGTACCTT
TGCGAGATGTAAGGGCCGGACGATGCAATGCAATAATGTCCAAAAAATCGG
GGTCAGGTGCTACAGTACTACTGGAAGCATATGTAAGTGTTTCATCTATAGGT
TCATATGCAGGATTATCAAAGGTAACAAATGTGGAGGGATTACCAATAAAAT
TGGGATCTGTAACCTTTACCTGTTGATAGGCCTTACTATACAAACGAGGAGCT
GACACTCGTTGTATACCAGGAATAGGTGTACTACTAATAGGTTCTAATCCTGT
TCCATTAGTAGAAGCAAATACTTCCATTGGAATTTCTTCATAACTATGTGTGC
CAGCTGTGGACGTTGTAGTAAATATATGACCTGATGCTTCCCCGGCCTGTGGT
ATATCTATAGGGGGTTCAATATATAATGGATTTTGTATATTGGTACTTGTTAC
ATGTACAGTACCAGCAGAGGGGGTGATGTCCAACACAGCAGGTGTTGTTGCA
GAGGATGTGGTAAGTTCAAAGCCATTGCCACCACTAAATGTAGGTATAGGGG
CTCCAGATTGTATAATACTAGATTTCCTCCACCAAAGTAACTATAGAAGGTTCT
GTAGGACCCACAGGTTCAATAATAATGGGCGGACGGGTAGGGCCGATATCCA
CAACAGAGGGTCTACCACCCCTCCTAGGGGAATGTATCCAGTACGCCCCACC
AGACCCAGTTCCTGTACCTATACCAAGACCACCTAAAAATATTCCCAAACCA
CTCCATTGTAATATCTTATCAGCAAGGGTAGAACCTTCAATTTTAGGAATAAC
ATCAGGAGGGGCACGTACCAGCGGCCTTACATGTTTTGTATAAGTCTGTAGCTG
ATGCTCGTTTGCGTCGAGGGGCACGGACAGCTACCATTGTAACACGTTTATTA
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AGGTATAAAAAATAAGCAAATATACACAATATAGGTAGTTATATATGATGTA
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ACGCAAAGCACAAACACATAAACTATAAAAGGAATAATGATTACAGGTATAT
AGGTGTGTTAGCAGCACATATTGGCTTGTGTGTTGGTAAAATATACACTATAT
TACAATGACATTATTCCCAATGTTGAAGTTATACTTTGTGGTATTTTAACAGT
TGTTAAAAAATTATTACGTTGTGTTATACTGTAAAGGTAATTGTTACAATGC
CTTGTTGATTGGTATCATTACTGGTCCAATGCCAGGTAGATGATACATTGCAA
TACAATCCTTTATGCTTTTTAAATCTATATCTTAAACATTTTAACTATTTGTA
TCACCTTTTAGGTGTACTATAAACACAGTTTGGTCACTACTCGTGTCCCTTCCC
GGGTGATGGCCTCCACTTGTACTGTGTAGGTTGTTGTTTGTATAGTCCACTGA
CTGTCCGGGCTGTGTGGCAGCTGTGGTGACAGTGGTGACTGTGGTGACGGTG
GTGTCAGGTCCACTGAGTCGTCGTCGCTTTCCTGGGTATGGCGCCTCGGTTTC
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CTGGCTCAGCAGTTTTAGCAGTGGATATTTGGTTGCTGCTGCAGGTA TAGAT
ACAAATTCAGGACAACAGATTACCTGACCACACACATGTACAGCCCATTGCA
CACCTGTCCCATATTTTTCCGCCTCTTGTTTAAAGTCCACGTAATACTGCTTAT
ATGCCCCCTTGTTGTATAATATATCCCTTTTGCATCAACATCTCCAGTACCTTTAC
ACCACCCTATATCAGTTTTATAATACACATATTTCCACCTAATATAATCCATT
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CAAGCTTCTGTATTATAGTCCGTGTTTATTAACGACTGCAATGCTATATGTA
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TTGACCCCCCTGACTACACACACTAGGCAACCCGCCATTTTCTTCATATTGT
TTATCTACCTGTACGGGCGTAAGAGTTTCCACTTCAGTATAGCCATACCCGCT
GTCCGGTACACTGTCCACGGCTCTCCTTTTGGCCTGTGAATTATTTACTTGGTG
TGTATTCTGCTGACTGTGCTTTTGTGCTGTGTTGATTGTTATGTCTTGCAA
CGGGCTGTTCTGACTACCTAGTAACTTTGCTTTTAAATTGCGCACTGCCTTTGT
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CACTACTATTGTCTGTTTCATCATCTGATATTGTGTCCCCTGTATGTTTTTCCA
CTATAGCTTCTACTGAAAACCACCCTGTACACCCCCGCCCTCCTCATTGTGA
CCTTCACAGTCCATTGCAGGTTTACTGTGCAGCACACTGATGGCACACCAAG
GACACGTCTTCCATTAACATCTGCTCCAGCACTCGAACGTTCTGTGCTGACTGCT
CTGCACAGCTAGCTGCACTATACTATTACACATAACAATTGTGCTTCAATTC
TGTAACACACTTCTTGTCCAGCTTGTCTGGCCTGCTGGTCACGCATATTATCT
GTTTCATCCTCATCTGAGCTGTCAAATTGTTTCATAGTCCAATTGTTTCGTAGCA
GCGTAGGTCAATTTCCGGGTTGCGGCACCAGATCTAGTATTACATCTTCAATAT
TAATTATGTTTCCATGCATTGTTCTTTACACTTGTGTTTCTGTTTGGCGCCTTG
GTCTCCAACAATTTGTACACAACCCTTTCCACTGCCCTGCTATTTTCGTGAAAT
CGTCGCTTTTCATCCACAATTCTTTGTTTTTCTTCTGGCCCCAATGGCATTGGA
CATCTATGACACCTTATTAACAAATTACATAAACTTTTTTTAGTTAAGGCTTCT
AATGTTGCACCATACACAGAACATGTATAGCGTCTATACTCTGTTATTTTTGA

ATAAAATATTACACATCTTTTGCATGCAGCATACGGACTCCTATCTCTATATA
CTACTCTTAGGTCACAAATTGCAAAATTATATACATCAGCCCATTGTAAGGTT
TCCTTGCAATATACACACTGTACCTGCAAAATTTTGCAAAGTAGTATTCAAGCT
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CTGTTTTGCACGTAGCTAGCCTTTTACTTTTATATGTACCGGTTTCGGTTGCAA
CCGTTTTTCGGTTACACCCTTTTAGTAACATATAATTGTAAAAGTTATTATAA
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GCTGTCCTCTACCAACTTCAACACCAACACAGGCCACACCAGGCGTTCTGT
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FIG. 9**HPV73 Deletion Probe Template (SEQ ID NO: 16):**

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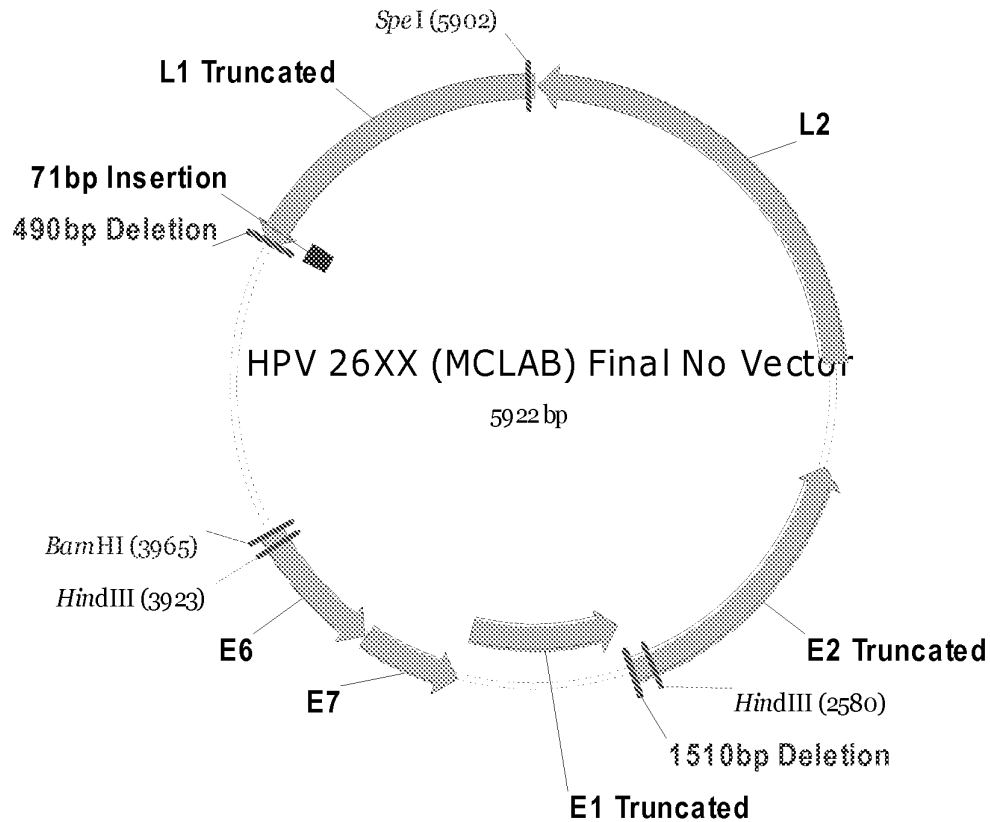
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TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG
GATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAA
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FIG. 10



HPV 26XX

Truncated Probe (SEQ ID NO: 117)

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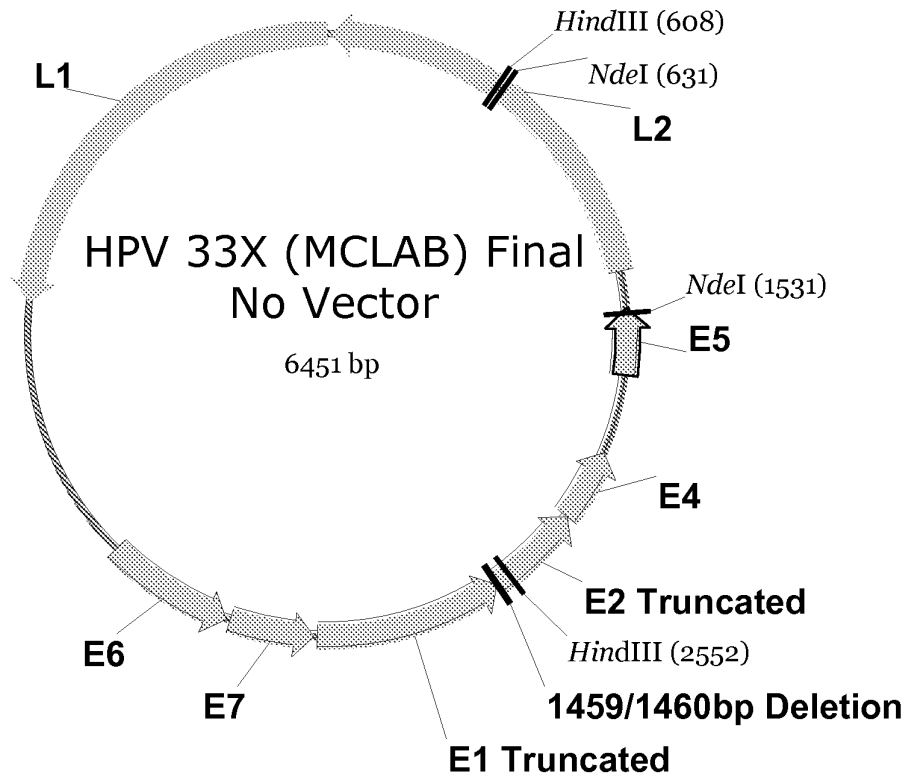
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ACCACCCTATATCAGTTTTATAATACACATATTTCCACCTAATATAATCCATT
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CAAGCTTCTGTATTATAGTCCGTGTTTATTAACCGACTGCAATGCTATATGTA
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CGATATCTTCCACTGAGGCATTTGACCCCCCTGACTACACACACTAGGCAAC
CCGCCATTTTCTTCATATTGTTTATCTACCTGTACGGGCGTAAGAGTTTCCACT
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CTGTGAATTATTTACTTGGTGTGTATTCTGCTGACTGTCGCTTTGCTGTCTGTG
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TAAATTGCGCACTGCCTTTGTATTGCGCTGTTTTTGTGTGCTGAAACAATGC
CTGGGTACCTCCTGTTCTGCATAATCACTTATACTACTATCATCTATAAATCC
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GTCCCCCTGTATGTTTTTCCACTATAGCTTCTACTGAAAACCACCCTGTACACC
CCCGCCCCCTCCTCATTGTACCTTCACAGTCCATTGCAGGTTTACTGTGCAGC
ACACTGATGGCACACCAAGGACACGTCTTCCATTAAACATCTGCTCCAGCACT
CGAACGTTCTGTGCGACTGCTCTGCACAGCTAGCTGCACTATACTATTACACAT
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GCTGGTCACGCATATTATCTGTTTCATCCTCATCTGAGCTGTCAAATTGTTTCAT
AGTCCAATTGTTTCGTAGCAGCGTAGGTCAATTTTCGGGTTGCGGCACCAGATCT
AGTATTACATCTTCAATATTAAATTATGTTTCCATGCATTGTTCTTTACACTTGT
GTTTCTGTTTGGCGCCTTGGTCTCCAACAATTTGTACACAACCCTTTCCACTGC
CCTGCTATTTTCGTGAAATCGTCGCTTTTCATCCACAATTCTTTGTTTTTCTTCT
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GCCCCGATAATAATATATGCCGGTGCGAGTTACATATTCATCCGTGTTGACAA
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FIG. 11



HPV 33X

Truncated Plasmid (SEQ ID NO: 88)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 97)

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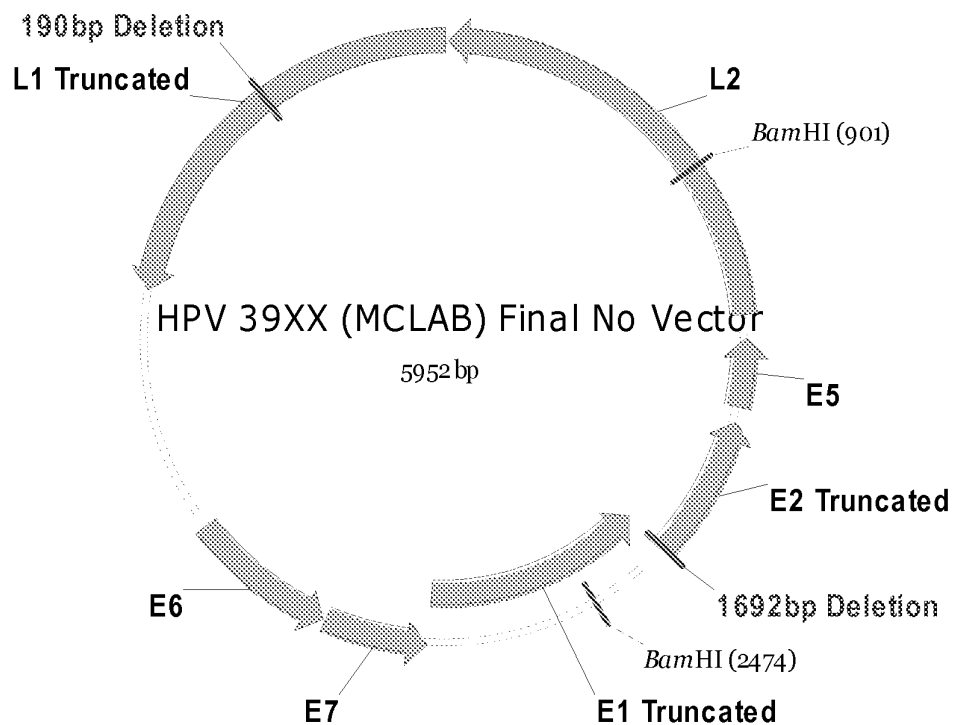
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CTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCT
TTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCA
AGCTGGGCTGTGTGCACGACCCCCCGTTACGCCGACCGCTGCGCCTTATCC
GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGG
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CAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT
CTACGGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATTTTTGGT
CATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGA
AGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCA
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TAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGGCTTACC
ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCA
GATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGT
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TGTGGTCTAAAGGCACAATAGGACTTAAATCCTGATAATAATGTATTCTAGCT
CCAATTTGTTTACCCTGCGAGTTTAAAGTGTGGCTTTTTGACCTACTCTACTA
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TATCTAGAAAGTCAGGATCAGGAGCAGGTGATATATCACTATGTTGAAATTG
TAATGTGTCTTCAGGGTCAAAGCTTTCAAATGCAGGATTATCATATGTTATAA
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GTGCTTGATGTTACATTACTACTGTCTGTGGAAACAACAAGGTATCCATTGG
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CAGAGGCTTCTGCAGGCGCTGGAGGGTGTAGTACAGATGGTTCAGTAAATGT
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FIG. 12



HPV 39XX

Truncated Plasmid (SEQ ID NO: 89)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 98)

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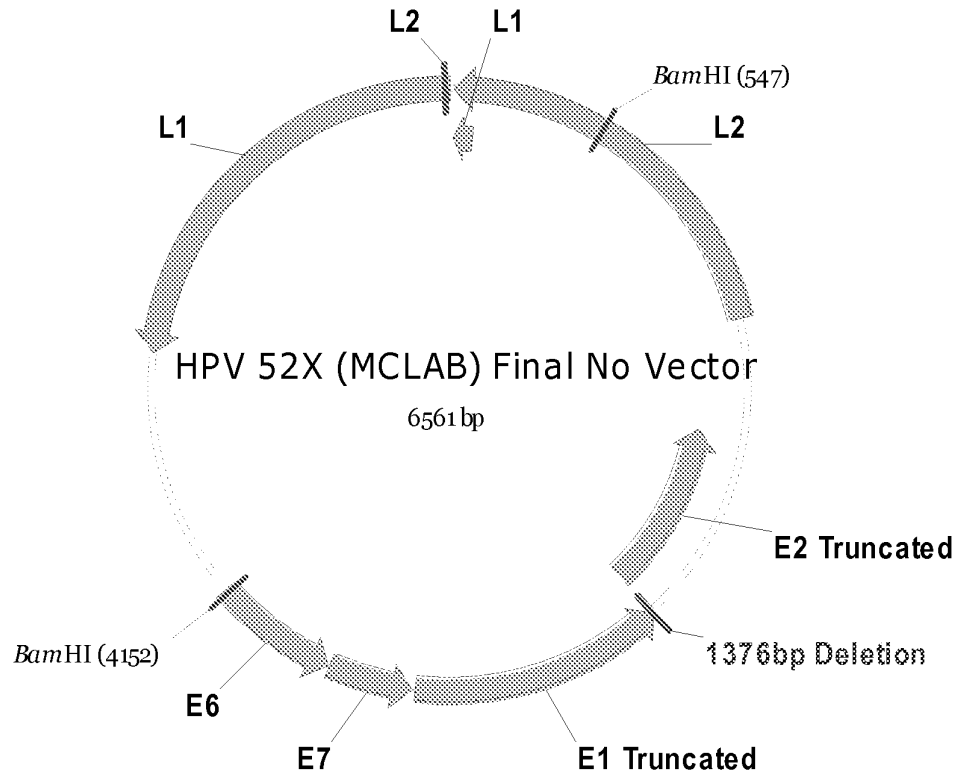
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FIGURE 13



HPV 52X

Truncated Plasmid (SEQ ID NO: 90)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 99)

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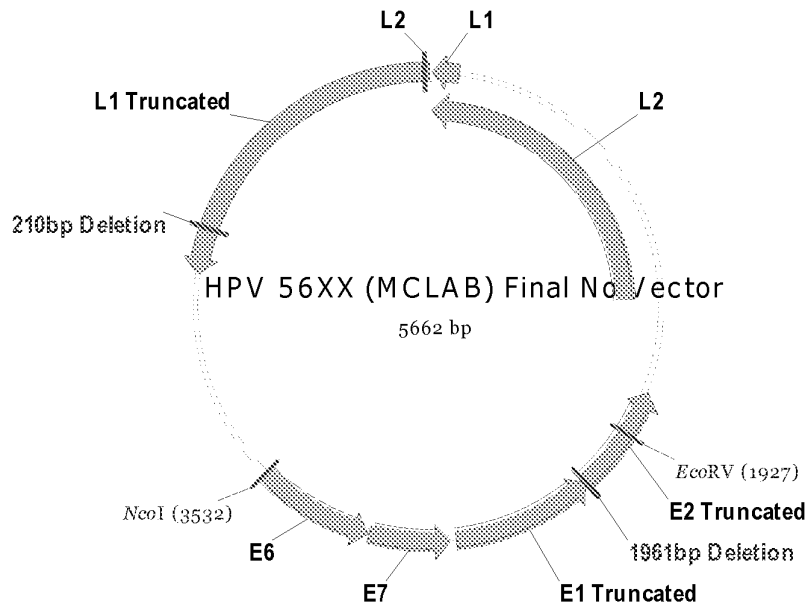

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FIGURE 14



HPV 56XX

Truncated Plasmid (SEQ ID NO: 91)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 100)

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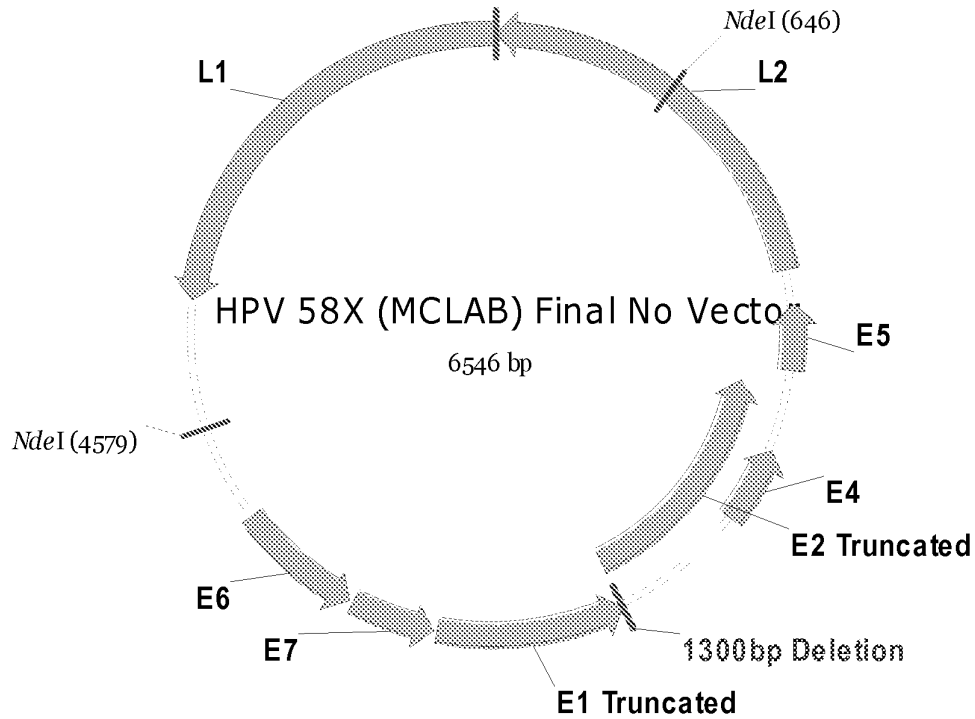
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FIGURE 15**HPV 58X**

Truncated Probe Sequence (SEQ ID NO: 101)

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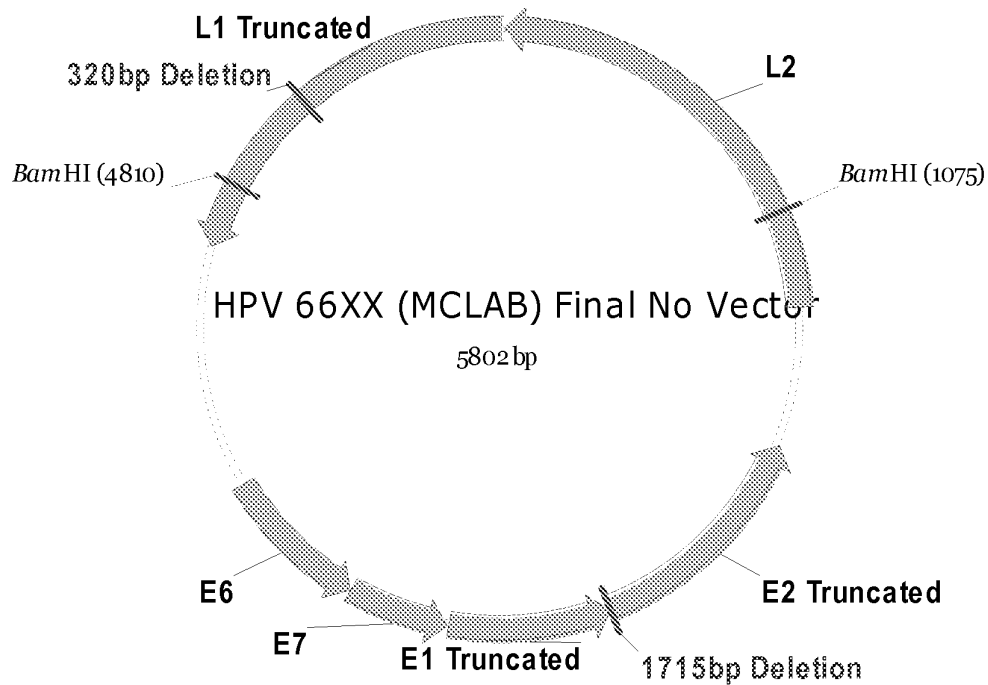
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FIGURE 16**HPV 66XX Plasmid**

Truncated Plasmid (SEQ ID NO: 93)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 102)

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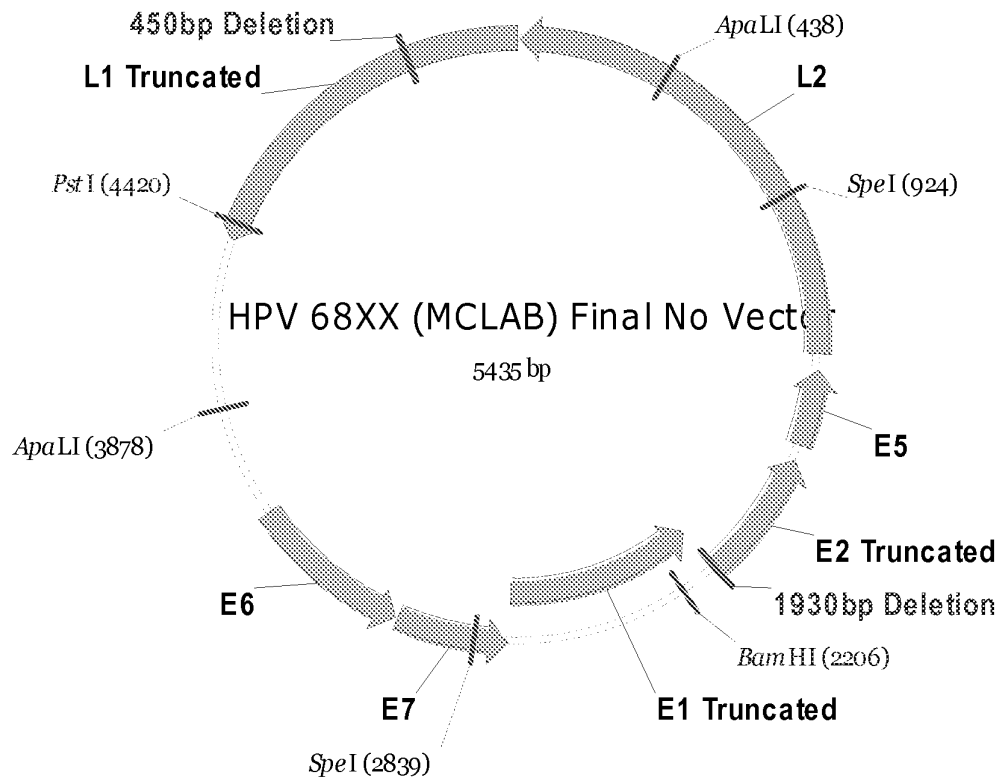
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FIGURE 17



HPV 68XX Plasmid

Truncated Plasmid (SEQ ID NO: 94)

Truncated Probe Sequence (highlighted) (SEQ ID NO: 103)

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ACTACTAGGAGTTTCACGAATGTCAGTGCCCTTAATATACATGTCAGTGGGA
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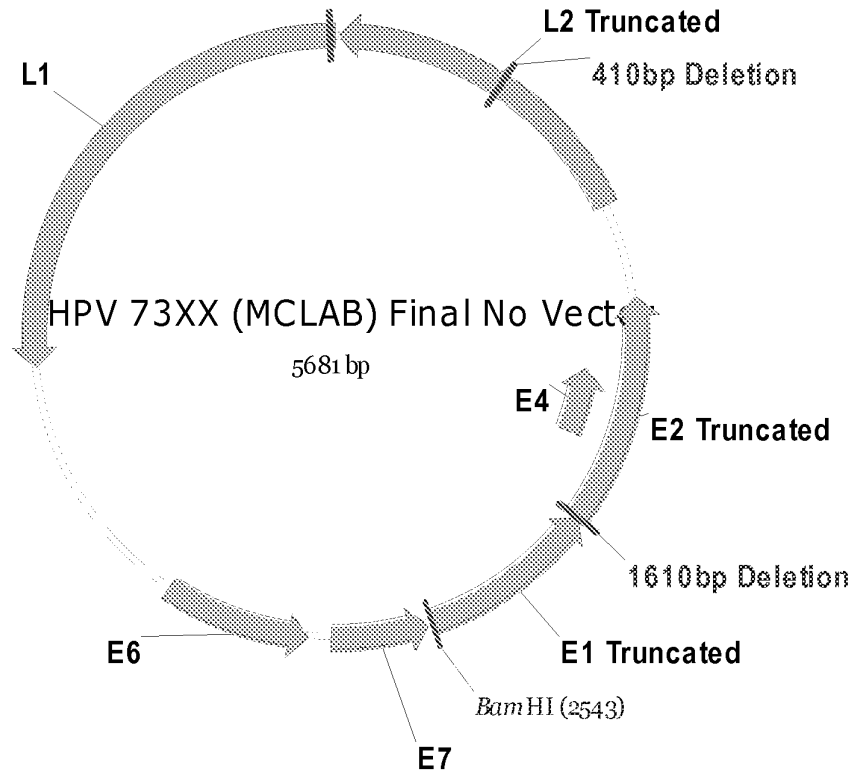
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GAGGGGGGGCCCGGTACCCAGCTTTTGTTCCTTTAGTGAGGGTTAATTGCGC
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CAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGG
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FIGURE 18**HPV 73XX**

Probe Sequence (SEQ ID NO: 95)

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 TGGGGCACACAATACCTAGTGTACCCATAAGCAACTCTTCTATCACTCTTAAA
 TCAGCTTTGTTGCTTTCAATGGCAAGGCATACTGTGCACTGACACTTCGTGCA
 GTCAGTAACTATTCTGTAACACTCTCGTTCAGCTTGTCTGTCTAGATGGCTGT
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FIG. 19

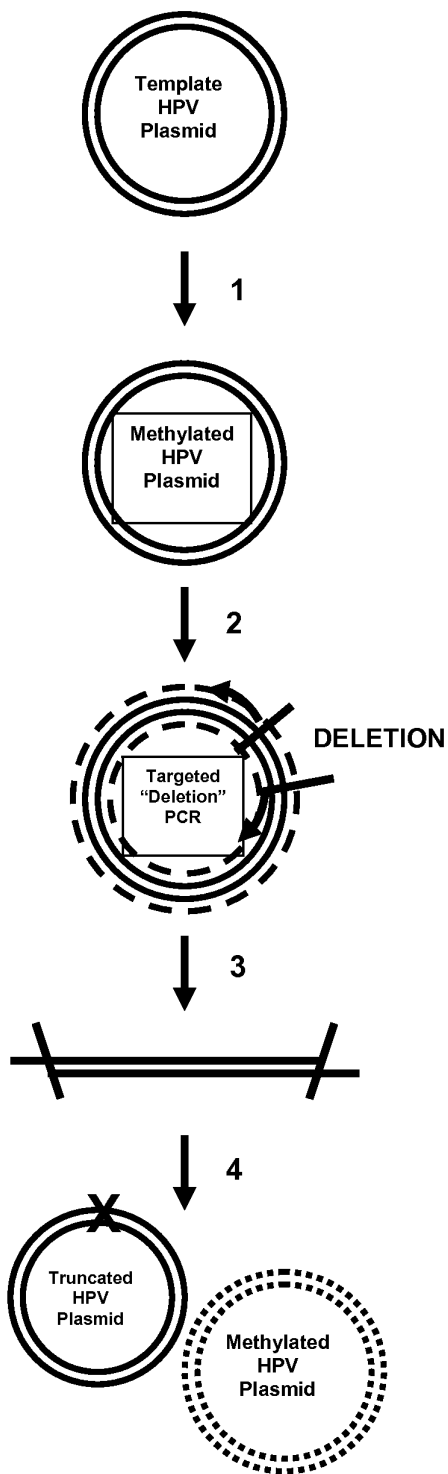


FIG. 20

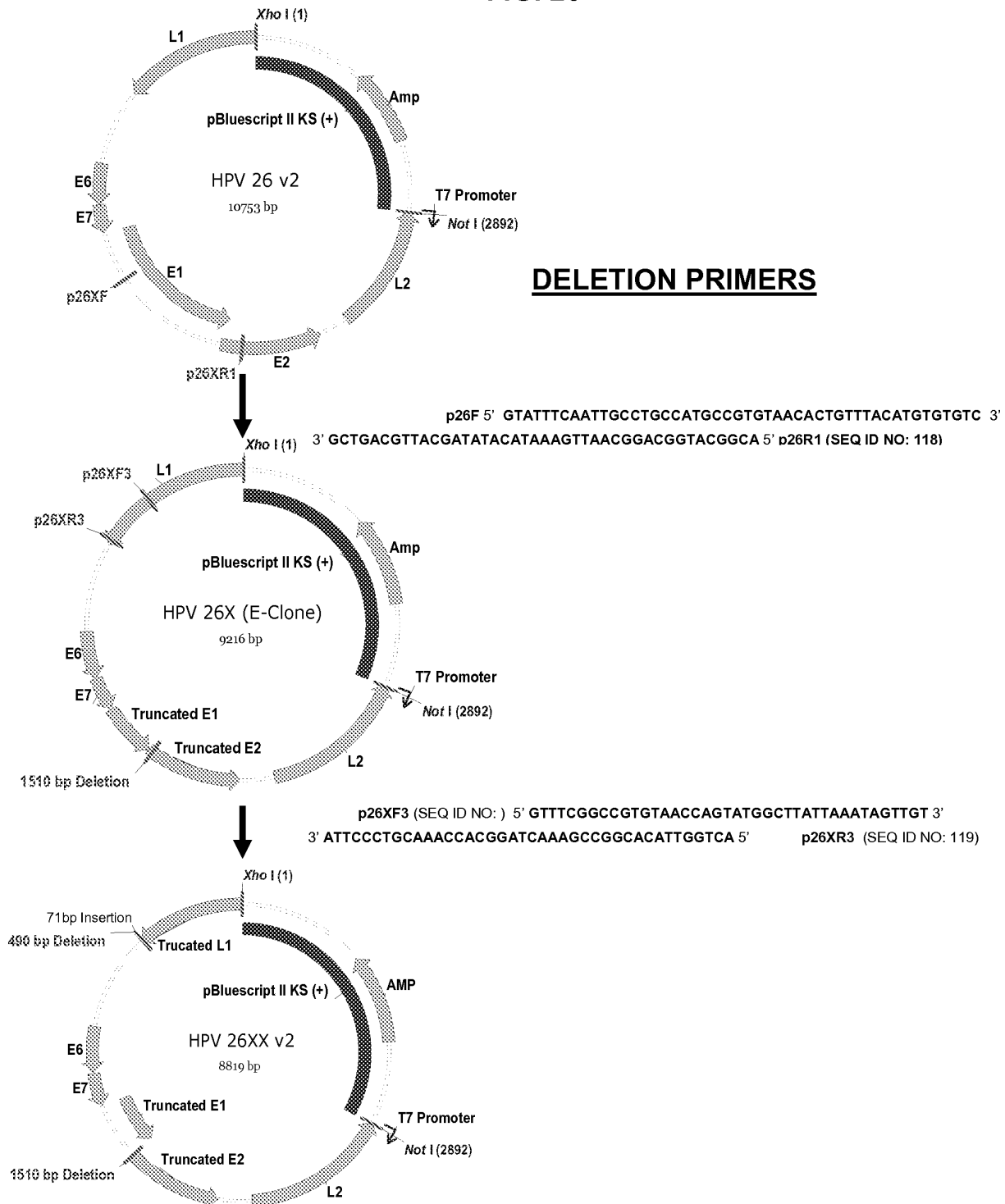
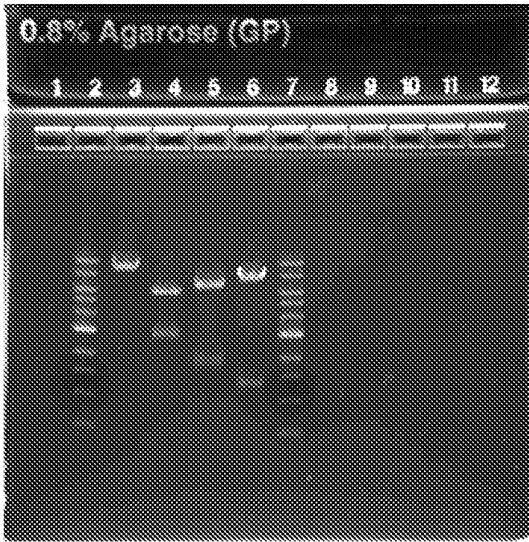
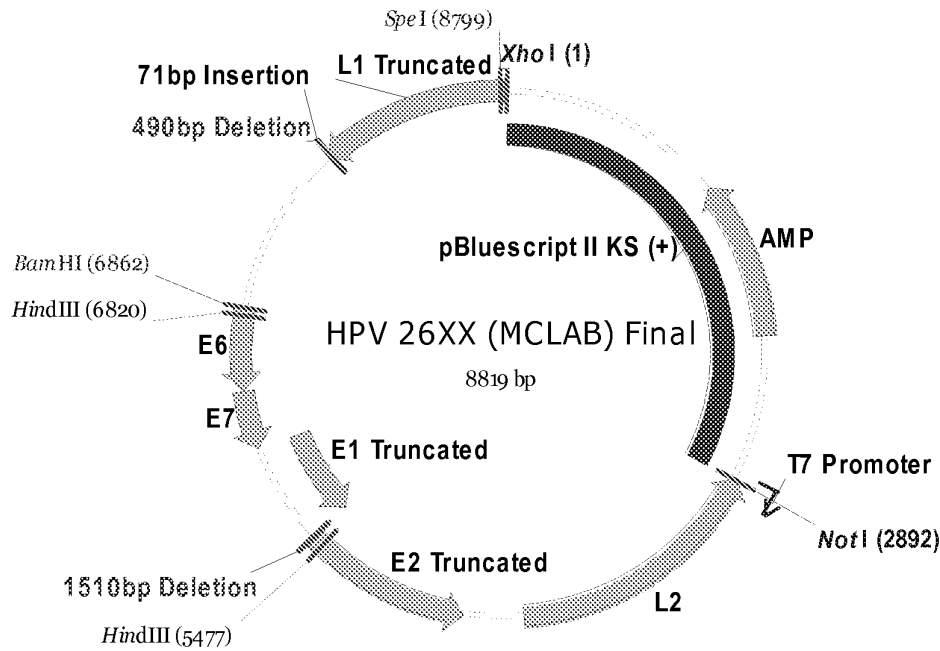


FIG. 21

HPV 26XX Restriction Map Verification



HPV 26xx		
Lane	Digestion	Fragment Size (bp)
1	Blank	-
2	1 kb DNA Ladder	-
3	<i>XhoI</i>	8,819
4	<i>XhoI/NotI</i>	5,927 + 2,892
5	<i>BamHI/SpeI</i>	6,882 + 1,937
6	<i>HindIII</i>	7,476 + 1,343
7	1 kb DNA Ladder	-
8	Blank	-

FIG. 22

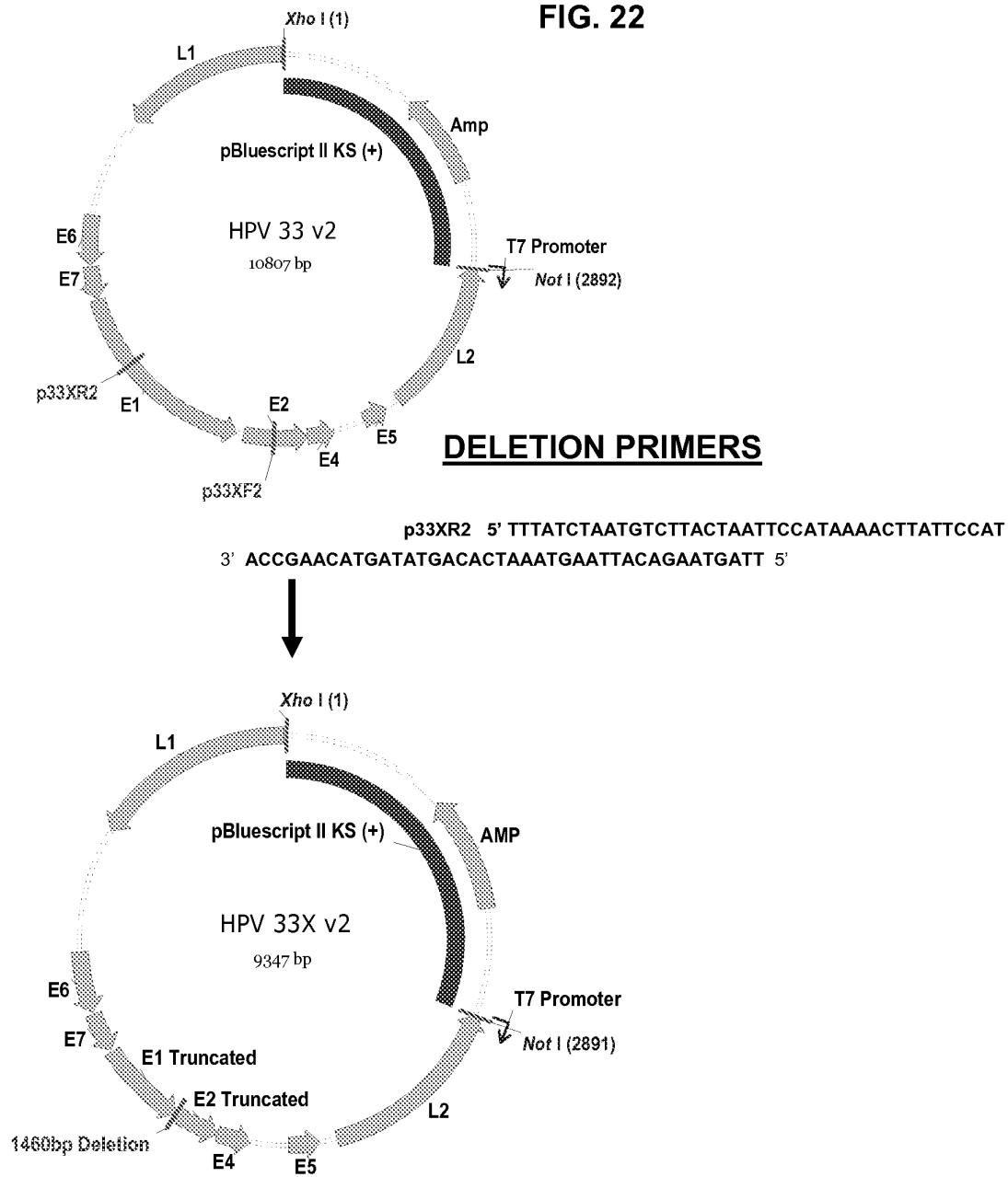
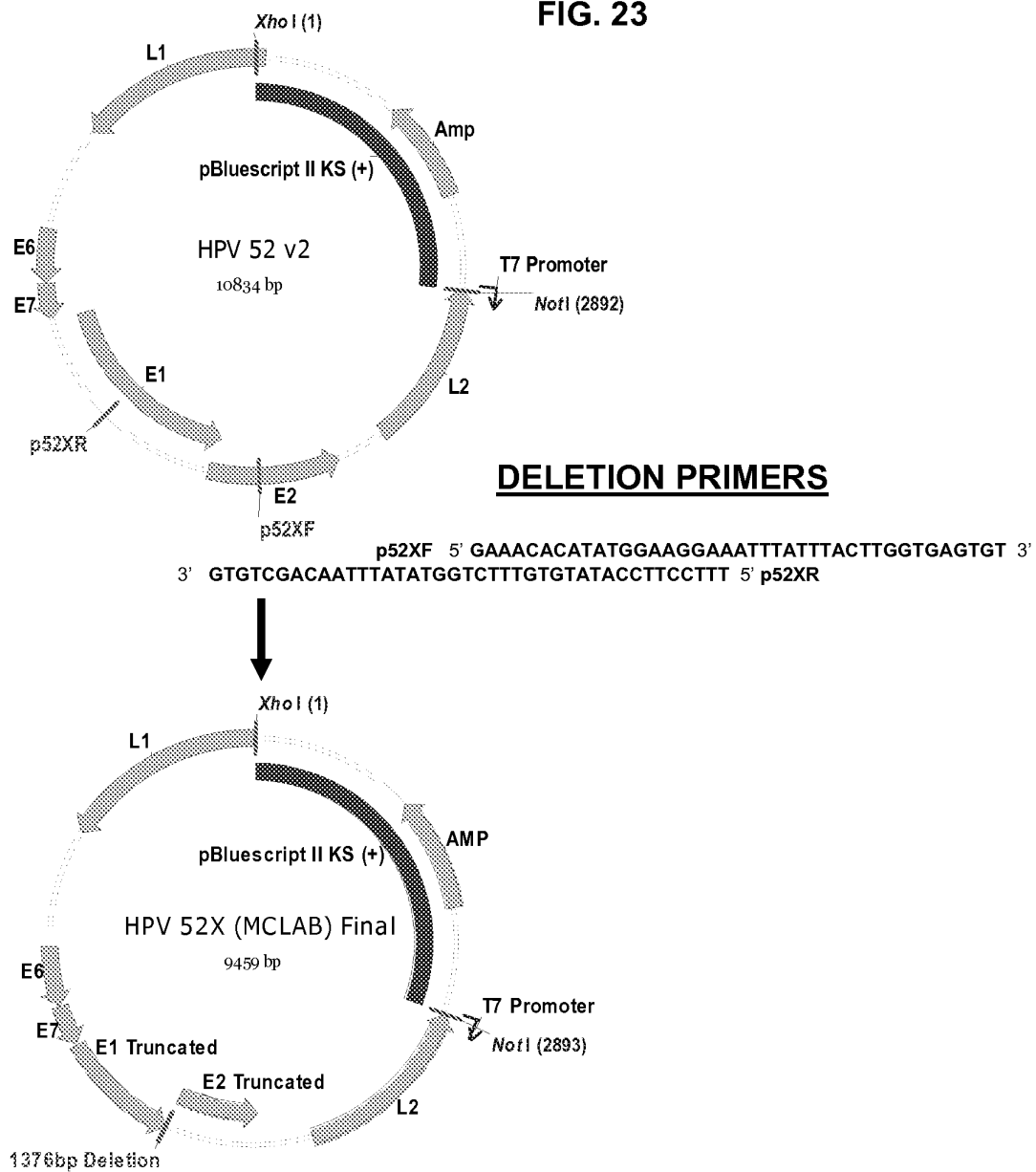


FIG. 23



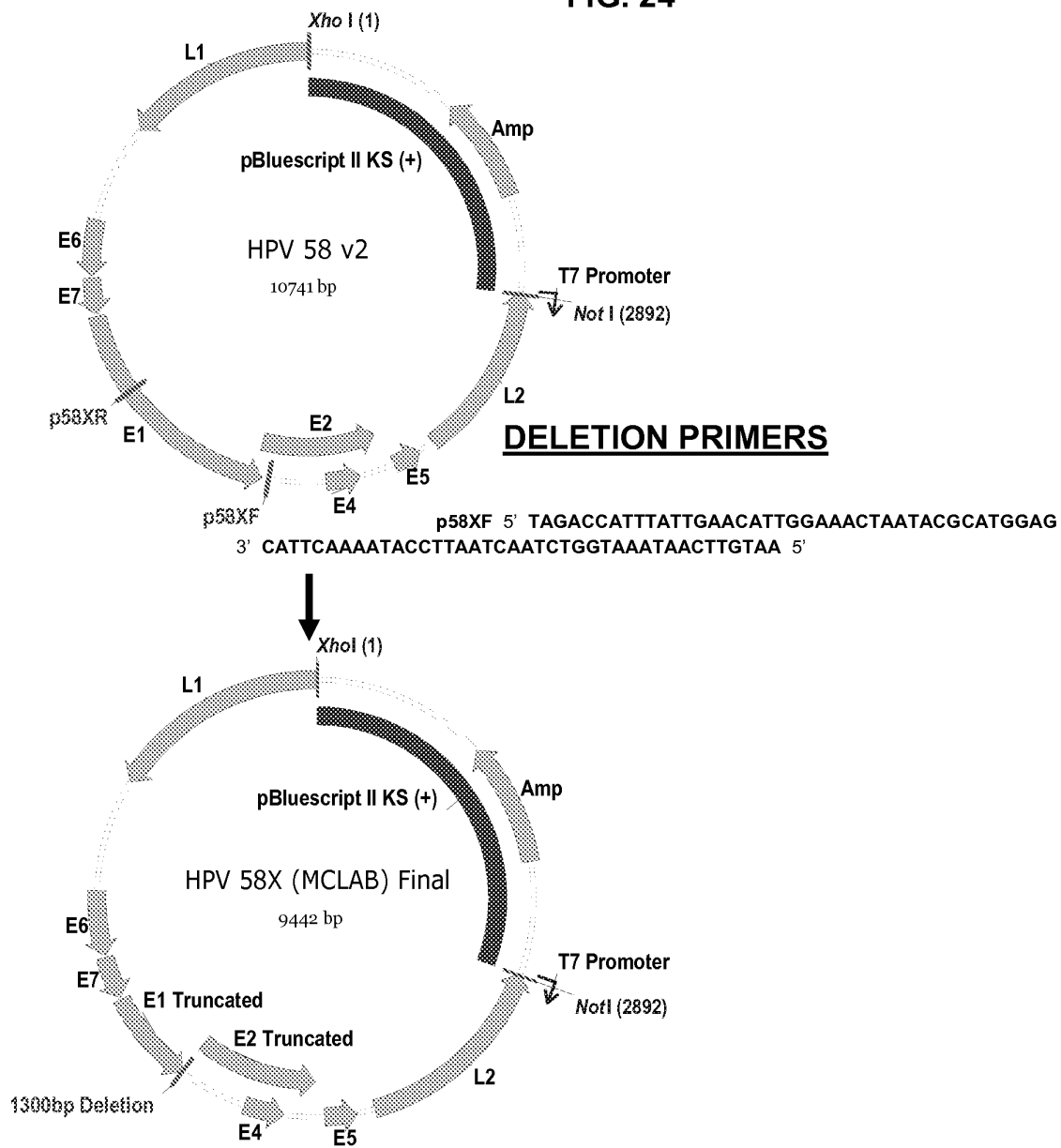


FIG. 25

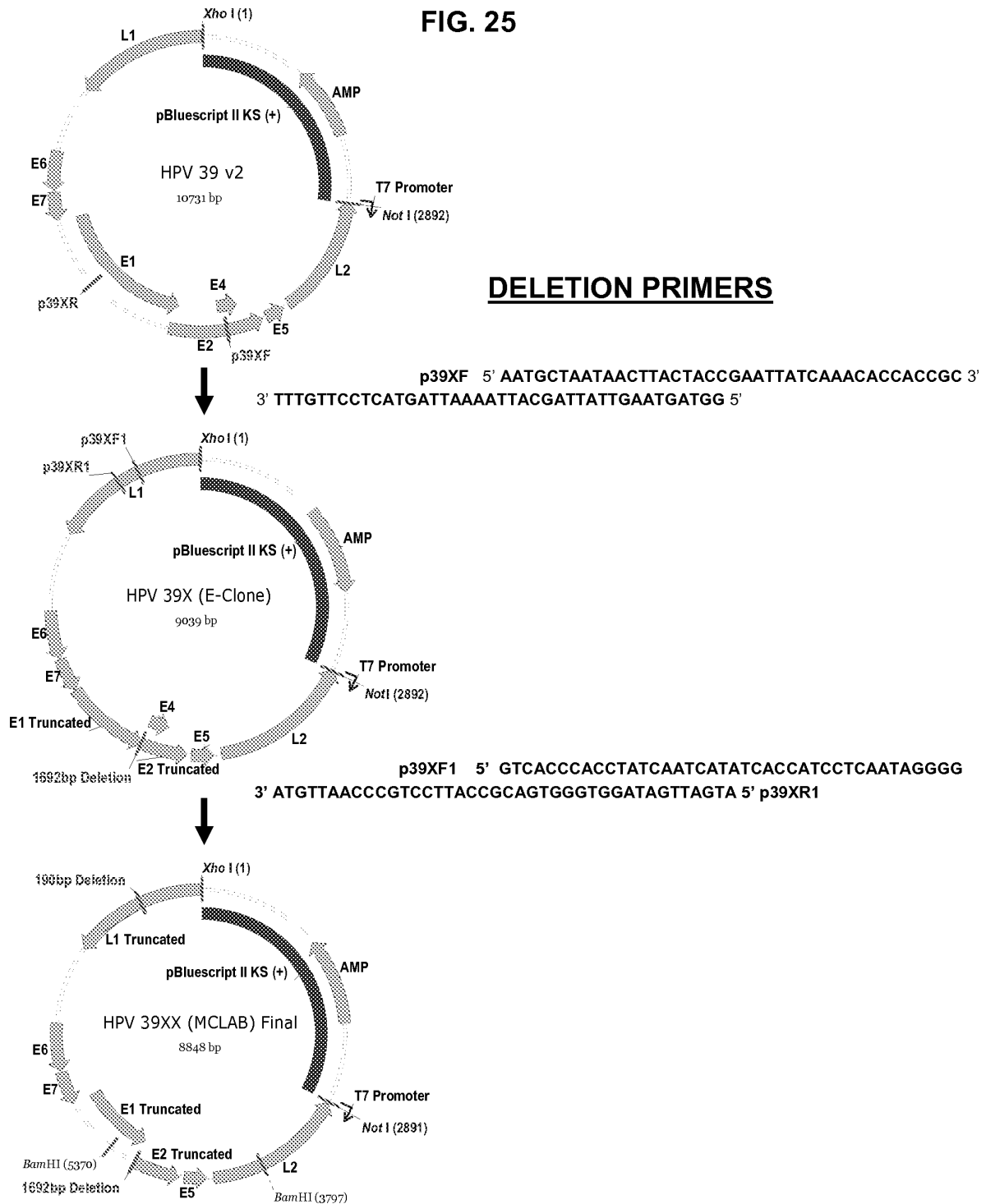


FIG. 26

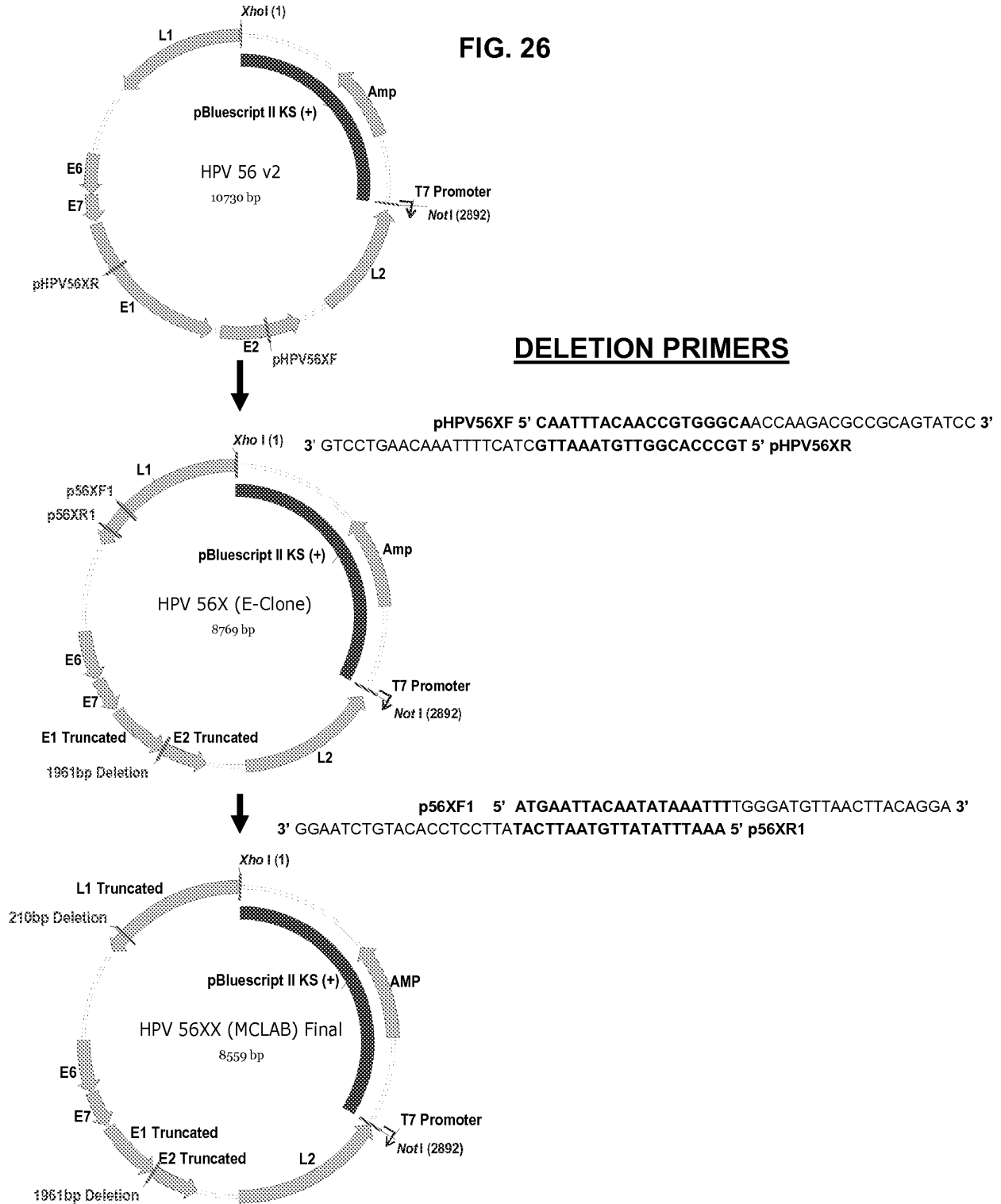


FIG. 27

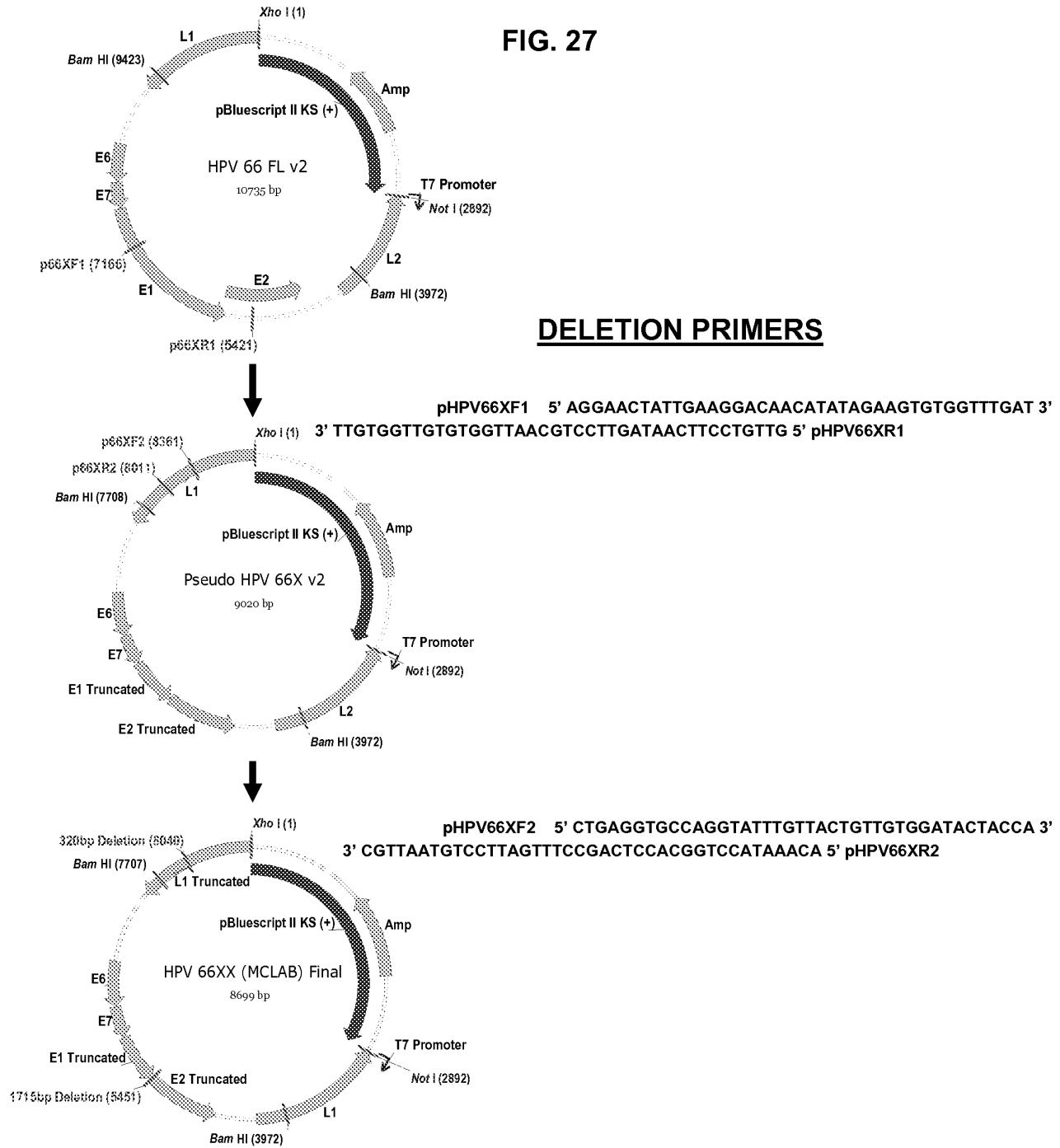


FIG. 28

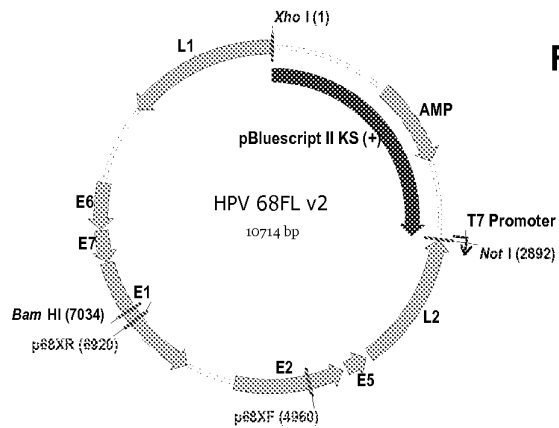
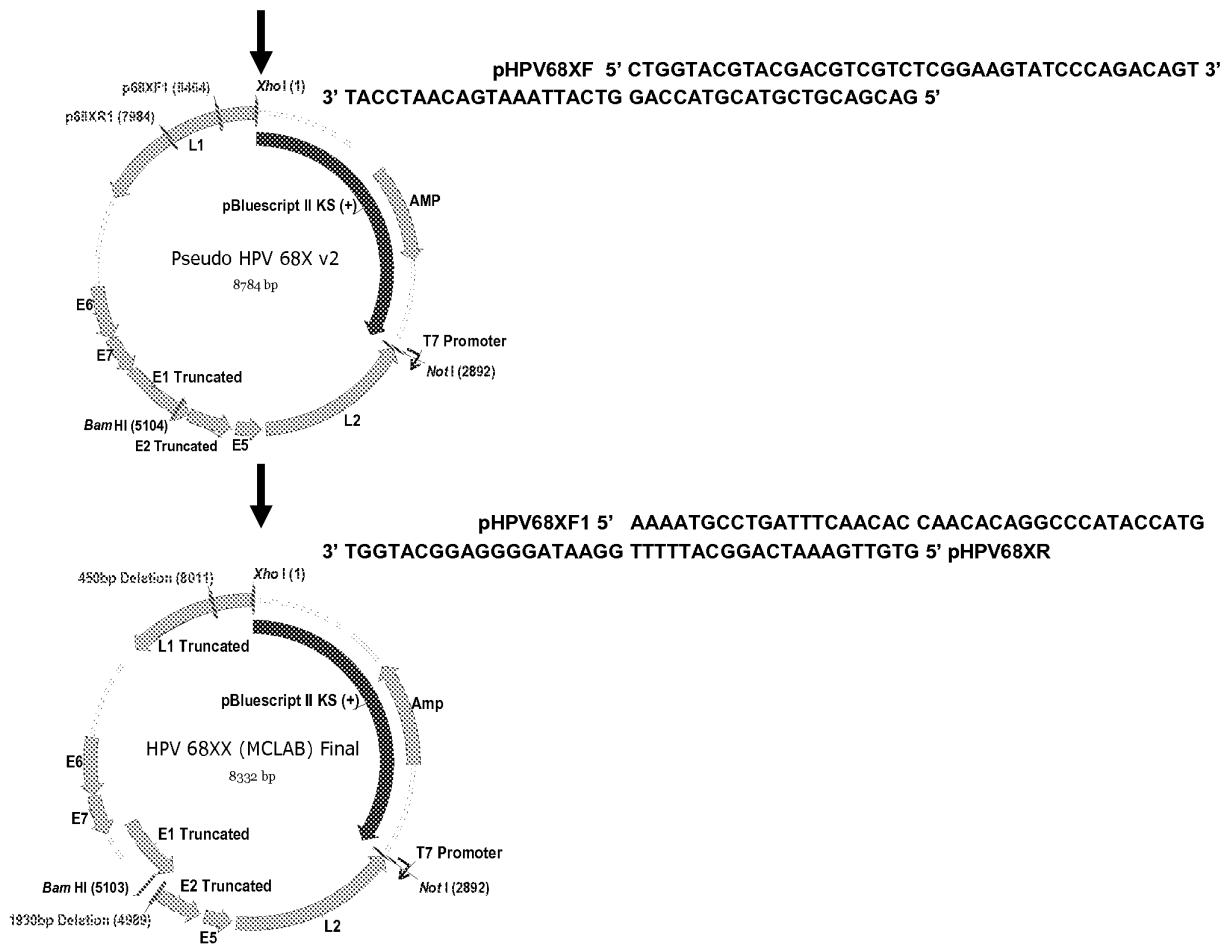
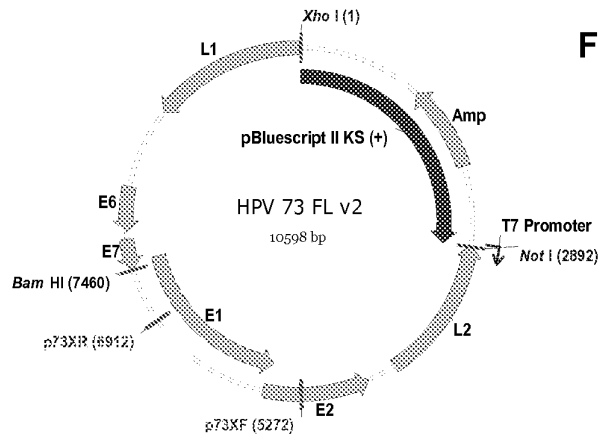
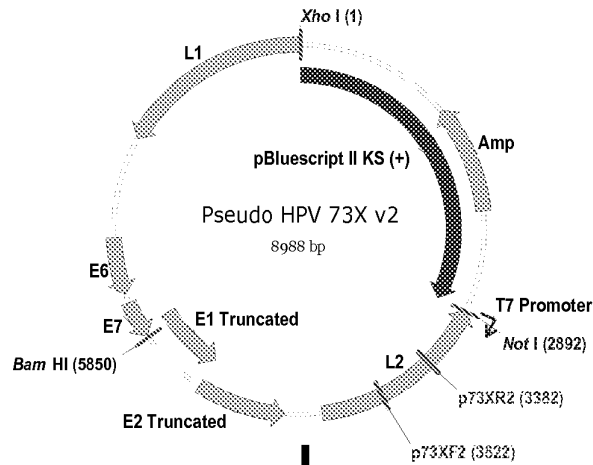
**DELETION PRIMERS**

FIG. 29

**DELETION PRIMERS**

pHPV73XF 5' ATTGCTAGCACAGTAGAGGTTAGATATGACTGTGAAAAGG 3'
3' TCCTTATTACGTTTCGTCGTAACGATCGTGTCTCATCTCCA 5' pHPV73XR



pHPV73XF1 5' TTCCTCTAAACTATCATATCACCATCCTGTATAGGGGTGT 3'
3' CTACAATGTATAGATATACAAAGGAGATTTGATAGTATAG 5' pHPV73XR1

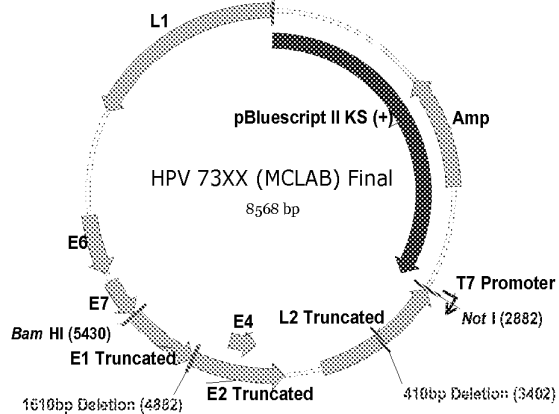


FIG. 30

		HIGH RISK																	
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GenBank Accession Number	HPV Type	gb HPV 16	gb HPV 18	gb HPV 26	gb HPV 31	gb HPV 33	gb HPV 35	gb HPV 39	gb HPV 45	gb HPV 51	gb HPV 52	gb HPV 56	gb HPV 58	gb HPV 59	gb HPV 66	gb HPV 68	gb HPV 73	gb HPV 82	
NC 001356	gb HPV 1	49	48	48	49	52	49	48	48	48	48	47	52	49	48	49	49	48	
NC 001352	gb HPV 2	54	56	55	55	55	55	56	56	55	54	54	55	56	54	56	54	55	
X74462	gb HPV 3	56	57	56	56	56	55	57	56	57	56	54	57	57	56	57	55	56	
NC 001457	gb HPV 4	50	50	49	49	51	49	50	49	50	49	47	52	50	49	50	50	50	
NC 001531	gb HPV 5	48	49	49	49	50	48	48	49	48	48	45	49	49	47	49	49	48	
AF092932	gb HPV 6	56	56	54	57	63	56	54	55	54	54	56	62	55	54	55	57	54	
PPH8CG	gb HPV 8	49	48	48	49	48	49	49	49	48	48	44	48	49	48	50	48	49	
M14119	gb HPV 11	60	59	58	60	53	60	59	59	58	60	53	54	58	58	59	60	58	
X62843	gb HPV 13	58	58	57	58	60	59	57	57	56	58	56	60	57	56	57	58	56	
X74474	gb HPV 30	60	62	65	60	62	60	61	62	65	60	71	61	62	76	61	60	65	
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X74478	gb HPV 40	57	57	56	57	58	57	56	56	55	56	54	58	56	56	56	57	55	
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AJ620205	gb HPV 43	58	57	56	58	59	58	56	56	56	57	55	58	57	56	56	58	56	
U31788	gb HPV 44	58	57	56	58	59	58	57	57	56	58	56	58	56	57	57	57	56	
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AJ620209	gb HPV 81	56	58	56	56	57	56	57	57	57	56	56	57	57	56	57	56	57	
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AF293960	gb HPV 84	55	57	57	55	57	56	56	57	56	55	55	57	57	56	57	55	56	
AF131950	gb HPV 85	62	72	63	62	64	61	73	72	63	61	61	64	71	64	73	61	63	
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RISK

LOW

IDENTITY

% IDENTITY

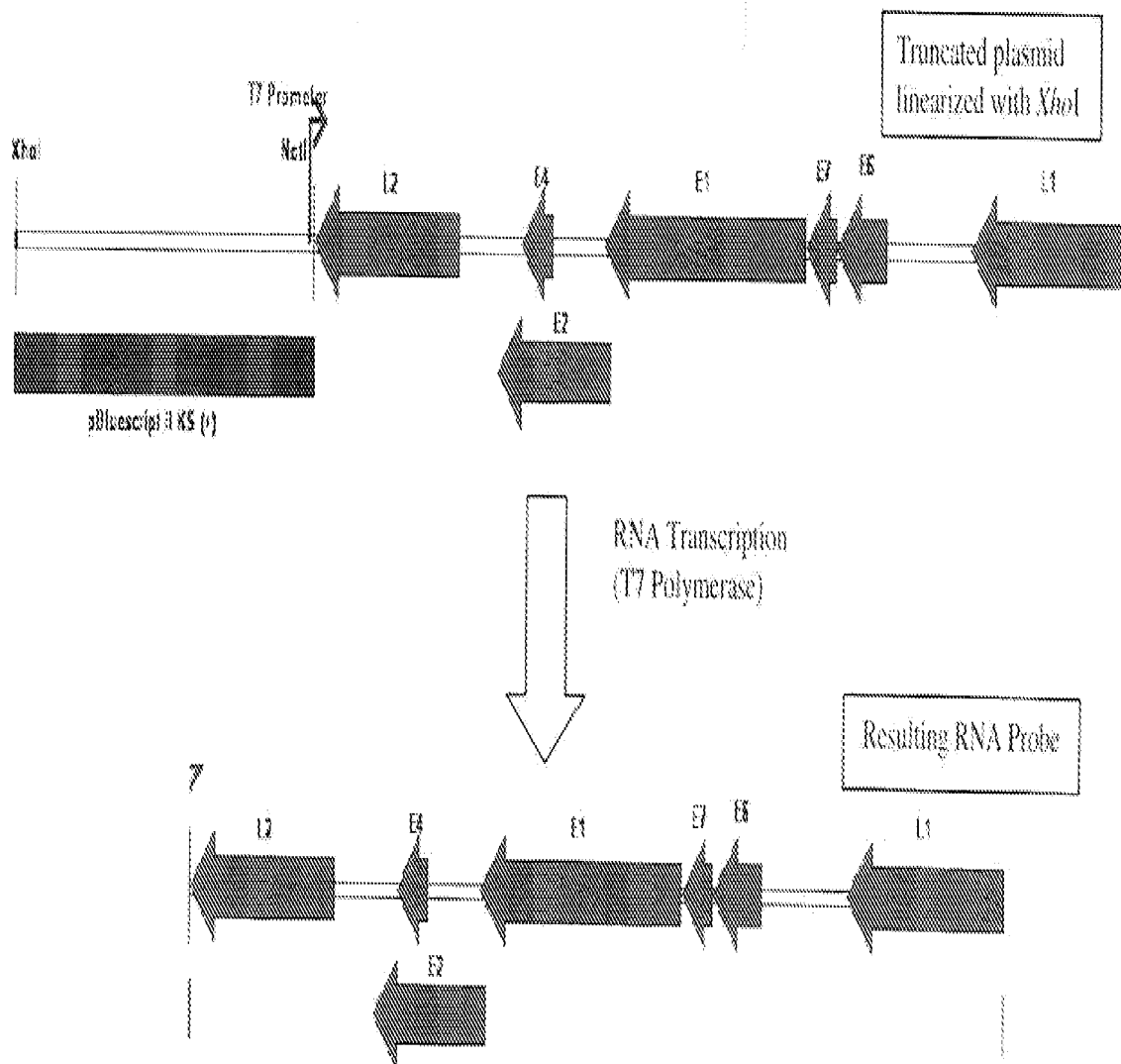


Fig. 31

071001_ST25
SEQUENCE LISTING

<110> Qiagen Gaithersburg, Inc.
Mallonee, Richard
Qiu, Peter

<120> FAST RESULTS HYBRID CAPTURE ASSAY AND ASSOCIATED
STRATEGICALLY-TRUNCATED PROBES

<130> 2912943-071001

<150> 61/347,941

<151> 2010-05-25

<160> 119

<170> PatentIn version 3.5

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<213> Artificial Sequence

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ttttcgggat ttatgcatag tatatagaga tgggaatcca tatgctgtat gtgataaatg	300
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<210> 45
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 <212> DNA
 <213> Human papillomavirus type 33

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 <212> DNA
 <213> Human papillomavirus type 33

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<213> Human papillomavirus type 39

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<210> 48
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 <212> DNA
 <213> Human papillomavirus type 39

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<210> 49
 <211> 1376
 <212> DNA
 <213> Human papillomavirus type 52

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<210> 50
 <211> 1961
 <212> DNA
 <213> Human papillomavirus type 56

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<210> 51
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 <212> DNA
 <213> Human papillomavirus type 56

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 <213> Human papillomavirus type 66

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<210> 55
 <211> 1930
 <212> DNA
 <213> Human papillomavirus type 68

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 tgtgcatcct gcataaacct ttcgtaatag gttttttgtt gttcatacat ataataaca 240
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<220>
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 <223> Deletion Primer (Human papillomavirus type 33)

 <400> 63
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 <210> 64
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 <220>
 <223> Deletion Primer p52XF (Human papillomavirus type 52)

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<400> 69
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<223> Deletion Primer p39XF1 (Human papillomavirus type 39)

<400> 70
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<210> 71
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<400> 71
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<223> Deletion Primer pHPV56XR (Human papillomavirus type 56)

<400> 73
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 <400> 77
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<400> 85
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<223> Deletion Primer pHPV73XF1 (Human papillomavirus type 73)

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cncngaaang	nnancncng	nnngnnnnnc	nannanngcc	ncnacnnca	accagccngn	3360
acancncnc	cnnngccc	cngnaccnnc	agggnccnc	anngcagggn	ngnnnanagc	3420
cngncacagc	cggggcacac	aacnngnaan	ngcccaaca	gcannngcng	nagagnacga	3480
aggncgncg	cagngcnang	aangcanagc	cgnagngngc	nancacaacn	gngacaanan	3540
gncacaangn	agnaannngcn	ngnggcnnng	ncngcnngnc	cancnggccg	gnccacacca	3600
ncngnanccn	ccncancnga	gcngncaccn	aanngcnan	agcagnagnag	gncagnngnn	3660
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cgnnggacaa	annanacanc	naanagnnan	nncacnnaan	ggnnnnnnna	ccncncnnc	3900
naangnnnnnc	ccanacagng	aanannngana	angccnanan	ncacnnancn	naganaaaaa	3960
gcgnaggcac	anaanacaca	cgccanangg	annanngncn	cnananacna	nncgnaaanc	4020
ngnaaanaga	aacnngnana	ccncncnncg	nngnagcnch	nnnnngcacn	gcacacacng	4080
cagccnnann	ncangcaccg	anncnccag	caccncacac	aanncgngca	gggnccgggg	4140
ncgngngncn	gganccnca	acanggccgn	gcgnnagcna	cacngngnnc	nanananana	4200
caccggnnnc	gngncgaccg	nnnncggna	caccnannnn	nnnacnagna	naagannana	4260
annnanaann	aaaaaaaaa	gnggngngg	gnacggnaac	cgnaaccggn	cngnagngc	4320
acaccnggng	agnaacagna	nnnaancang	nnnnacaaga	canannnggc	gnaggangna	4380
cnnngngaag	ccaacacngn	aannagngca	nngaaagnnn	aaanaaaana	ngngccnaaa	4440
agcagnnnna	aacangcaan	gnnaggcaan	gnanngnnna	gcngcacngc	aggaccngng	4500
agncagcaag	aagncagnnn	aggcgggaca	acaagnagnag	accagnggac	ngcaggaaac	4560
caaagganna	agcagnnngc	ngaaacnnan	anaaaaanag	nngnnacngn	gccaaggaca	4620
accaaagnng	ngccaagacc	aaccgaannc	ggnnaggann	naaaangng	ganagnacaa	4680
aanggnacaa	aanggagagn	anaaggcnaa	nnagnagnng	aacaaagnag	gacacagggg	4740
agggcagggg	acacagggna	gggcaggaga	ngcaanagan	naggagcaac	ngnanngnna	4800

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cncangnggg	ngcaaccana	acanaagacn	acnanaaana	gnnnagnaana	nacagnacnn	4860
nannaanaca	nacacaaaac	anacanacag	gaacngacaa	anacaacaaa	cangcacaca	4920
canacanaac	angcaaacaa	cacagnacac	acacaaaaca	annacanaac	aaacaanggn	4980
naccnnnnnaa	ccngngnnng	acanacacaa	cacanacang	acacagacaa	nnaccaaca	5040
gacaanggnn	accnnnnnaac	cnnnnncnnc	nnngnggagg	nacgnggggc	cgangangca	5100
gggcgnnnnna	gnnnngggccn	agccngnagc	ccngccngna	acaaaaacnn	ccnaccnaaa	5160
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nccnnnaaag	gancnccnn	nccnnnaggn	ggngngnnnn	nnngacaagn	nanagcagna	5280
gaagngacaa	ancngnangn	gnccnccaaa	gangcagacg	gnggnggggn	aaggccaaan	5340
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aangnaannn	ngcacaanng	aaaaanaaan	ngnaaancaa	annccncgcc	angacgaagg	5460
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ccannanngn	ggcccngcgc	acgnngnaac	cagnacggnn	nannaaanaa	nnggganncn	5640
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gcccnannaa	aaaagngncn	aacaaacann	ngcncacgnc	naagaaaaaa	gaacaaacng	5820
ncaccanang	gcncgcnagc	cannngcaaa	naancnggan	acnnacanac	acngcnacan	5880
anancaangg	gcacancacn	nnnacnagcn	ngcaaggnan	naaaanccan	gcaaccaaen	5940
ccngnancna	ccangncccc	anccngnann	acacngnnna	ngagcngnag	gggaggacaa	6000
nccccaggan	nccngaann	annannacaa	ggggnncccn	naccccaang	nncaccnana	6060
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cnannancna	naccaggnnn	accagcanan	nngnnacngg	nnncagnanc	ancaaacnng	6180
nnnaanaaag	gangcccacn	aanaccacaa	ccnaaaggcn	gncccnacc	aannnccaag	6240
ccngnacagg	cccacaccaa	ccnnnggggn	ncnggggnan	aaaaagangn	ancnggaaaa	6300
ccaaannnnan	nagggncggg	caannnaann	cnaaanaccc	ngnanngcag	gccagacacc	6360
nngggaacna	aaacnnnnnn	accannacca	cnacnggngn	nnnnaanaga	aaaanaggga	6420
ngnccnacng	nnagnaancg	agaacngccn	gcanaanaan	agangcnngn	gcgagacaca	6480
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<211> 5662

<212> DNA

<213> Artificial Sequence

<220>

<223> Human papillomavirus type 56 double truncated probe

<220>
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 <222> (1)..(5662)
 <223> n i s u o r t

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 caaagggcca cgnacngggg ccngnaggca acacnangnc aggaccngaa naaaangng 180
 nnncccacac annaccnaaa ggggcagnna cannagnggn gnnacnagca aaagacaang 240
 nggaaggcnn nanagгнааg ngngcagaag gngnagcaac ngacnggcna gacaaaccag 300
 gngcnncanc ancnanannn gcanaaanан cananaggcc ancaaangaa nnannngcag 360
 acaanaangg cngcannnca annnccncag ccngngcaan aggacnnana ncanaanaan 420
 aangcacacg ggcacnann ngngngccnc nacgngnnng nanagnagcc nnnngccaa 480
 gccnacnaaa acgnacacca ccccnacng ngagnaaangc aggccnangn aangcnacna 540
 nanncanaaa ancaggggna ggagccacac ccgacggaga aaaagcnaaa gangngncag 600
 naccnncaaa aagngganna ncagcagana cnaangnngc aggnananc agaaangcag 660
 ggncagnnac cnaaacngc ngaaangcnn nncnananaa ncnaggagcn gcaanacgcc 720
 naaagccngg aanaggagna cnacnaanag gnnngnacc agaaccngga acagcaaang 780
 nngcanagg nannncnca nagcnangna naccagangn gggngngcna annaaaanan 840
 ngccagacac cncgccngnn ngnggggccc caanaacagg gggancaana aanaacggan 900
 nggnnanang ggnacnacng acangnacag nacnagagn nggngnaana nccaacacgg 960
 caggngnagn ngngangag gangnaannn caaanccccc agaccagna aaannaggaa 1020
 ncccngcacc aganncnana acacnggacn ccncaacnaa ngnaacaang gaaggngcng 1080
 naggcccnac ggannccaca acaanaggng gncgcgccgg agnnacanca acnannngg 1140
 aaggccnaga cccaangga acanagccng cacgaccccc agaccagnn ccngnaccaa 1200
 ngccaaggcc nccaaaanan gnaaaaaaac nccccanng caanannnna ncagcccang 1260
 nnnnnngcnc nannnnanna acaacanccn cnggacangn accagacaac nnacangnnn 1320
 nananagnng ngngcagan gcgcgnnngc gncgngnggc acggngggca accanagnag 1380
 naacagnacn acnacannna canannannn annanccaca aagcangnaa anananacac 1440
 agcacaggac agnaaaaaaa nancaaaaan annanaaang nannaaaaaa ggangnggcn 1500
 anaacaaacc aaaacaanan nannaanaan anacaacngg aaaacacaga ggcaganagc 1560
 aaaagcggga caacangaca cacaagcaaa caaacacaaa agcaaagcgc aaacacacca 1620
 anaacacaaa aancaanggn ngcaananan angcgnagca gcaangnang gcaggnacac 1680
 nncacgnann ngngngnnac aanaacacaa angnananac aanananaca gcacnnnang 1740
 gaaaacncan nngncccaaa cnaaccngna cacnacnggg aannnnnaca ngacnnaaaa 1800

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cngnacnngn	ccaanganan	gnngangnna	cancacaaaa	caangnnnna	nannnnngaa	1920
ancganancn	acaacannnn	aancngnnag	gnncaccnnn	naaangnacn	acaggcgngag	1980
ncnnancacc	aggngggngg	nngngngnga	nacnncnacn	ncnacngncg	gnanngncng	2040
ngncgcngan	gngngngngn	gnngngacac	acnnngcgng	ggacncncng	gaggagncaa	2100
anncngannc	ccgnagncng	ggncgnnnnc	cnggncngng	gganacngcg	gcgncnnggn	2160
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anngnnncna	ncaanancca	nangnannac	agagnccnca	cnanngnnac	nanaggnacn	2280
gnnnngngag	ccnccannnn	gngnannccc	gcacccacgn	cccngnaccn	cnncancnac	2340
cngnnncggn	gnnnccagag	nnnccaangn	anngccanac	ccgcnngcnn	gnaggncnga	2400
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naanggacna	gcnananacn	nncgnnnnag	nnnnngcaac	gncngnnnan	cngcangngc	2520
ngnnngnacn	ngcaacaann	gnngacngnn	ncngcgncng	ccngnanann	nngnananan	2580
gaancgncna	naaanccanc	naaancngna	ncnannncan	ccnccncgnc	acnnncanca	2640
ncnganannn	nancncngn	nnnnnnnncn	acaanngccn	cnacnncaaa	ccanccacaa	2700
cancccnnc	ccnccccanc	ngnaccnnca	ggngacgcca	nngcagnnag	nnacnngang	2760
cgagagngg	gcacggnnacn	gnaaacgcac	ccanaagcag	cngnngnaca	acacgcaggn	2820
ccnncnnngn	acncngaang	nccaacngca	ccacaaacnn	acacncacaa	caaggnacgn	2880
gnannaggna	acacgnangn	ngnnnagcnn	gncnagcnng	cngnggccgc	nccngcaaan	2940
ggncnacnnc	anccncancc	ncanccncng	agcngnccaa	nngcncanng	cacngnaggn	3000
caannncngn	nngaggngnn	agnncnaana	caacgncnng	cagcgngngn	acnnnaccan	3060
gcangannan	acngnagann	cncnaggnn	ncnagangnn	ngncnccagc	accccaaaca	3120
ngacccggnc	caaccangng	cnannagang	aaancgncnn	nnncngncac	aangcaanng	3180
cnnnnccncc	ggagnnaacg	gacnnngaca	ncngnagcac	cnnannaana	aancacanaa	3240
cngnnnnnna	gnnanacnnn	cnagnnagc	nccanacacn	gaanagncan	aanaccnana	3300
nnnnncnaacn	nnacnanaaa	acaanaaaca	nacncngcac	acngcanaag	gaaaancanc	3360
ccnanacacn	aannnnnaann	cagngcangc	aaaannanan	accncagcac	gngnnagnnc	3420
nnnnnnngcaa	nanacacang	anaanchaag	ancaannaaa	ggannnncna	anaccncacn	3480
caagngggngc	aggcnncgng	gacgnnccng	ngganngngg	aanngnggcn	ccangganang	3540
gnccacacag	aanaagcngc	cnnnnanang	naccgnnnn	ggncnnaaac	ccnnnncggn	3600
cacnccaan	ananaaaagn	anganngaaa	cnncacaaa	nnaaaagaaa	ccngnnnnng	3660
cacgaccgna	aacggnnnna	aaaaangagn	aanngnangn	nacangnnng	caaaacagan	3720
acngacagan	acnnggccna	canagnngan	ncngcaagcc	aaaacaccaa	agnngcagac	3780
acangnanaa	aaangngngc	ccaacagcac	naaagnangc	aganaaaagc	anagnnggca	3840

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gncanagnac	cngngagnaa	nacagggngc	ggnacngnac	anaanncaag	nanaaaacac	3900
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naanggcacn	aggccangca	accgaanncc	nnnnagnnna	aanaanggca	cnaggccang	4020
caaccgaann	cggnggcang	nacaaaangg	cggaaaaaca	acaaaangga	gnangnnang	4080
gnanananan	gnangcacia	nacacanagn	acacngnaca	caanaacnna	caaaacaaaa	4140
gccacaanaa	ngacacaccn	nagnnnangc	aaccacgcgn	aaaagcacnc	anncangnnn	4200
annacagaca	cacaaacann	acacananan	acagncanac	anacaangaa	nacacacaca	4260
aacannncac	acaaacacia	canacacana	caaagnanaa	aanaaagcac	anacacaaac	4320
anaccanana	nacaacacac	aaacacagnn	acacaaacac	acaacaacac	acnaccgcn	4380
nnnacgnnnn	gcnggngnag	aggnggaggn	aggagcagan	cgcnnnnnag	aggnagcnac	4440
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caggncngna	gaaaaacngn	ccngnaagnn	aacancccaa	aannnanann	gnaanncana	4560
nnccnccaca	ngncnaaggn	acngannaan	nnnncgngca	ncanannnac	nnaacngnnc	4620
ngnagcagna	cnaanagnca	ngnnagnach	ncnagnagna	ncnacnacag	naacaaanaa	4680
nngannaccc	cagcaaangc	cannannang	gccnngggca	cgnggcaacc	aanaaggnnn	4740
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acncggaggg	ggnnncnch	canngcnacc	cnnaaaanan	aacncngcag	gnanngnnnc	4860
cccaacnnna	ccagcccnan	naaaanaang	ncnggcaaan	aanngnnccc	ngcgnaagna	4920
aaaccacana	gaancaccan	aggcancngc	agacannnnn	aaanagncag	ganannnaca	4980
ggngganngn	acaangncna	aaggnaccnc	agccnnagan	nccngcaaca	ccnnaaagnc	5040
canagcgcca	aanccngngn	cnancangnc	cccancncn	anaggngnan	naannaangc	5100
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angnnacccc	anagcgggag	nacanccaac	aanacacac	ngngnnngcn	ngccancaac	5220
nganananng	ncccnacnan	cnncnanaac	annannannn	gcnaaanngg	aacnnncagn	5280
ancanccagc	cnannaaaca	angganggcc	acnnagccca	gcaccnaaag	gcngnccgcg	5340
gccnaccncc	aaaccnacac	angcccacac	naaccgnncc	nggnccggan	nanaaanann	5400
agnancngga	agcccaaach	nannagggnc	gggcaaccgn	accnnaaana	cccnananng	5460
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angnccnacg	gcaagcaanc	nggaacngcc	ngcanganaa	aananacnag	ngcgnnnnac	5580
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 <211> 6546
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human papillomavirus type 58X truncated probe

<220>
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 <222> (1)..(6546)
 <223> n i s u o r t

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 anaaanggac nagacanaga ngnnacagaa gangcaangn cnggaccagg nnccaangac 180
 acaagaggag ngncaaancc agnannnaan ggnanggaca cannacnggn acgngnggng 240
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 gcanaaaan canaaagncc ancannaana cnanaggag aaacagaagn annnaaagan 360
 ngnaanncaa anngnngcng cngnngnacc ngnnccngga caggcngnan gggacnnaag 420
 ncnnngnagn aangnacnnn agcccnann ngcnnnccac ngcgagnacg aagngnagcc 480
 nnnngcccaa cccnacnana acgnacagna cccngcgag aggnnaangc aggncngngn 540
 aangcaacaa nancnagaaa ancagganca ggagcaggcg anangncacn angnngaaac 600
 ngcaangngn ccncagggnn aaagccnnc aangcnggan nancanangn nacaagncna 660
 ngaggagang nnaaaaaagc agggncaca accnnaacnn gnnnggngnn gcgacngnan 720
 aaaccaaggc gngccacagg gcgagaccn ggaangggng ngcnagacgn gacanngcca 780
 cngncagnag aaanaacaaa ggnanccann ggnangnnnn canaacnang ngngcnaaca 840
 gnaggagagg aaanannaa angnccagag gccncgcag gngcaggagg gcggagnacg 900
 ganggcncag naaaggaggg annnaaangn gnagaaacag nnnngnanaga nganncncca 960
 anagaggaaa cannaagnan ngcaggngna gnancngcag agngngnaan ancaaaacca 1020
 ganggagngg gaanngangg ggcnggngca ccggcgncna naaaacnaga nccncnann 1080
 aaaganacaa nagaagaanc caaaggcccc acagnancaa cggnaacngg gggacgnang 1140
 ggcngnaaag gnanagccnc agacggnggg gnacnacaa ggggcacana nccagnccng 1200
 ccaccngnac ccgaccngn accaangccn aaaccncaa aaaacacccc naagcnacca 1260
 nancgnaana nnnngancngc nanagnagng ccnncacnn ngggananaac ancaggnggg 1320
 caggngccng aggcchnnga ngnnnggnaa agnnngnag cagangcacg cnngcgccnn 1380
 gnagaccggn ngngncncan aanaagacag ngcnaaaan aaaaagnan nnannagnaa 1440
 angnanaaaa anaanaaaaa caacacacac aacagnaaan annnacaana ccanaccacc 1500
 angngcagaa ccagnanaca gnnagnchnng nngggnaag nanngngcan gaaaannaan 1560
 acacancann ggnananana aaaanannaa gnaacagaaa aaaanncgna gagccgaccc 1620
 cacagacacc caaagcagca acaccaacac cagcaaccaa gcanananag aaanaganag 1680
 caccaanggc cgcaaaaaa ngcacaagca naaaaacagn aaaaacaaa caacaaaaan 1740

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ccnacnnngn	caccnnngnc	ancacnggnc	caangccang	nggangacan	annacagnan	1980
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aaangcacga	naggngaaac	nnnagaacna	cacacgnncc	gcccnnngna	ngnacagnna	2100
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ngngnacnnn	cgngngnggn	ggccncggng	gncnnngggn	cagcagnnnc	agnagnggan	2280
annngancac	naggnanaga	ngnaggacan	acaannaccc	gacnaccac	angnaccncc	2340
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gnngnnnccn	caanaanana	nannncacnc	caannngnan	aanccanngn	gnngcnnna	2520
ncannngcan	anngnacagn	nacngnnang	cnnnnnnnnn	naaagcannn	nngnggcncn	2580
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cngnnangna	gaanannacn	aanannnnngn	aanggaacag	nannacaacn	gncnacncc	2940
gnnncacngc	nnacancnga	acnagcccc	acccacnag	acnccgagnc	annnaagncn	3000
gcgncgcan	nnnggcnnnc	naccnggngn	gccancngcn	cagnnnccac	nncagnanng	3060
ccanancgc	ngncnncnag	cncaanaann	nnncgnnnnc	ngngngngca	nncnnnannn	3120
nnanannncc	acganacaca	cacannngca	gcccggncca	cacagnccnc	nacagcacnn	3180
ncngagcang	cngcaaacnn	ncgnnnnagn	gcacacacag	cannnananc	gnccaccccn	3240
nccngnacn	naaacaacgc	ncgggcngcc	ncngcnncng	ccngngnagn	acnnngnacn	3300
gaancancna	naaacncnan	naaancngna	ccacnancgn	cngcngnnnc	gncncanca	3360
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gacacaangg	ncnnngacaa	anaanacanc	naannaanan	nncannnaaa	cacnnnnnna	3960
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caagannngg	cannanagnn	nagnannana	naaaangnng	aaacangaac	aangngaccc	4380
aaaacgggna	gnccacaccn	ggcaanaaan	gnnagngagn	cacnnnnaca	agacanagnn	4440
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anaaaacaaa	caaacangna	naanaaaana	gngnaagnac	cacaacaann	annnnnnaac	5040
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ccanngnnan	gacnnngngc	acgcngnagc	caanaaggcn	nannaaanaa	nngnganncn	5640
gagгнааааа	nagagccacn	aggagnngga	aaaaangcac	nacnnnggan	aacngcagna	5700
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<220>
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 <223> n is u or t

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caaagggcca	agnacngggg	ccngnaggna	aaacnananc	aggaccagaa	naaaanggng	180
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nagaaggnnn	aanaggnaan	ngngcagaag	gngnagcacc	agacngacga	aangaaangg	300
gngccncanc	ancaanannn	gcanaaaan	cananaggcc	ancaaangga	nngncngnag	360
acaanaangg	cngcannnca	annncancag	ccngngcaan	aggacnnana	ncanaaanaa	420
aangcacacg	agcaccnann	ngngnacccc	nacgngnnng	canggnagcc	nnnnngccna	480
gccnacnaaa	acgcacaccn	gnncnacgng	nagnaaangc	aggccnangc	aangcaacna	540
nanccanaaa	ancagganca	ggagccacac	ccgagggaga	aaaggncaac	gnngngncag	600
caccnncaaa	aacagganna	ncagcagnna	nnaangnnng	ggggngngcc	aaaaangcng	660
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nngnnanagn	agnacnacnn	acangnacag	nacnagangn	gggngnaana	nccaacacag	960
caggngnggn	ngnagaagag	gangngaccn	caaagccccc	ngaccacagna	aaanngggaa	1020
caccagcccc	ngagnnaana	acacnagann	cnncnaccag	ngnaacaana	gaagganccg	1080
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aaggccnaga	gccnaagggg	acanagcccc	cccgaccacc	cgaccacagac	ccagnaccaa	1200
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nnnnnngcnc	naccnnanna	anaacanccn	caggacangn	accaggnaan	nngcangnnn	1320
nananaanng	ngnggcagan	gcgcgnnngc	gncgngnggc	acggngggca	accanagnag	1380
caaaaacgna	anacngnnac	aaanggnnna	nnaaccacaa	agcangaaaa	nananacaca	1440
gngnaggnan	anaaaaaaan	agnaaaaaca	gnanaaangn	ancaaaaaan	gangnagcca	1500
caacaaacca	aaanaaagna	annagnanaa	gacaannggn	aaacaaagan	gcaganagca	1560
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nccnccncgc	nnncancanc	ngananngna	nccccgnnn	nncnnncnac	aanngcnncn	3060
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ggaaacngan	cnaggncngc	agaaaagcng	nccngnaaan	naaccnccca	aaacnnanan	4860

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nnnagaaaca	gagnaanaag	ganggccaac	agcaagcaac	cnagagcnac	cngcangana	5760
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ngnnccaagc	agnaccaaga ggaanggnag nannagnana ngngnnggan gcagcagaag 240
ccaangaagg	aacnganang ngggaacgag nagnaaangn agcannanga aangcagnan 300
cnaanacngn	agnanngnca gnancaggng cananananc ananaagnna nccanagggn 360
ccgccngcnc	aggggcaacc aanggnngna gnncaangcn gncagcaggg gnaangnnac 420
naanancang	anaanagngc accngngccc caannngngn accncggcgn gnaaacangg 480
ncgccnnnnn	gccnacncng cnaaaacgna cngnnccncn ncgggangnn aaggcaggcc 540

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acaaggncaa	ccggcngnan	nncanngnan	ggacanagcn	cnaacacaa	nccngcacg	3060
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ngnaancnnc	gnnngnngn	naggngccnn	agnnnnncng	cnggacacaa	nggnnncagg	3240
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